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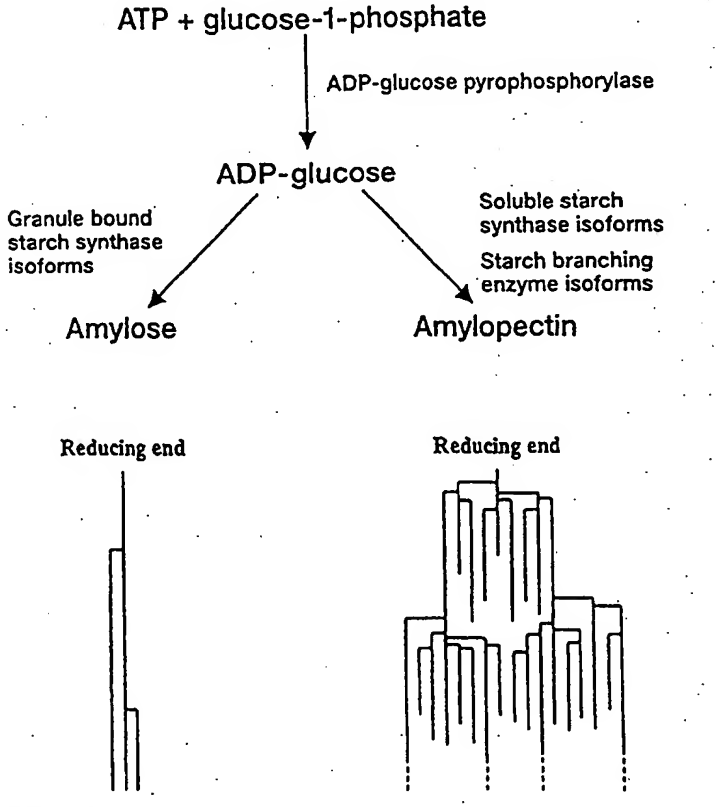
(54) Title: SENSE INTRON INHIBITION OF STARCH BRANCHING ENZYME EXPRESSION

(57) Abstract

A method of inhibiting gene expression is described. The method, which affects enzymatic activity in a plant, comprises expressing in a plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for a class A SBE intron in a sense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.



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SENSE INTRON INHIBITION OF STARCH BRANCHING ENZYME EXPRESSION

The present invention relates to a method of inhibiting gene expression, particularly inhibiting gene expression in a plant. The present invention also relates to a nucleotide sequence useful in the method. In addition, the present invention relates to a promoter that is useful for expressing the nucleotide sequence.

Starch is one of the main storage carbohydrates in plants, especially higher plants. The structure of starch consists of amylose and amylopectin. Amylose consists essentially of straight chains of α-1-4-linked glycosyl residues. Amylopectin comprises chains of α-1-4-linked glycosyl residues with some α-1-6 branches. The branched nature of amylopectin is accomplished by the action of *inter alia* an enzyme commonly known as the starch branching enzyme ("SBE"). SBE catalyses the formation of branch points in the amylopectin molecule by adding α-1,4 glucans through α-1,6-glucosidic branching linkages. The biosynthesis of amylose and amylopectin is schematically shown in Figure 1, whereas the α-1-4-links and the α-1-6 links are shown in Figure 2.

In Potato, it is known that two classes of SBE exist. In our copending international patent applications PCT/EP96/03052 and PCT/EP96/03053, class B potato SBE and a gene encoding it are discussed. In international patent application WO96/34968, class A potato SBE and a cDNA encoding it are disclosed.

It is known that starch is an important raw material. Starch is widely used in the food, paper, and chemical industries. However, a large fraction of the starches used in these industrial applications are post-harvest modified by chemical, physical or enzymatic methods in order to obtain starches with certain required functional properties.

Within the past few years it has become desirable to make genetically modified plants which could be capable of producing modified starches which could be the same as the post-harvest modified starches. It is also known that it may be possible to prepare such genetically modified plants by expression of antisense nucleotide coding sequences. In

this regard, June Bourque provides a detailed summary of antisense strategies for the genetic manipulations in plants (Bourque 1995 Plant Science 105 pp 125-149).

WO96/34968 discusses the use of antisense sequences complementary to sequences which encode class A and class B potato SBE to downregulate SBE expression in potato plants. The sequences used are complementary to SBE coding sequences.

Whilst it is known that enzymatic activity can be affected by expression of particular nucleotide sequences (for example see the teachings of Finnegan and McElroy [1994] Biotechnology 12 883-888; and Matzke and Matzke [1995] TIG 11 1-3) there is still a need for a method that can more reliably and/or more efficiently and/or more specifically affect enzymatic activity.

According to a first aspect of the present invention there is provided a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence partially or completely codes (is) an intron of the potato class A SBE gene in a sense orientation, optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in a sense or antisense orientation; and wherein the nucleotide sequence does not contain a sequence that is a sense exon sequence normally associated with the intron.

According to a second aspect of the present invention there is provided a method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron of the potato class A SBE gene in a sense orientation optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in a sense or antisense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is

affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

Preferably, the class A SBE gene sense intron construct is used in combination with a potato class B SBE gene sense intron construct as defined in PCT/EP96/03053. However, it may also be used independently thereof, to target class A SBE alone, or in combination with other transgenes such as other sense and/or antisense transgenes, for example antisense intron transgenes such as from SBE genes, to further manipulate starch quality in potato plants.

According to a third aspect of the present invention there is provided a sequence comprising the nucleotide sequence shown as SEQ. ID. No. 38 or a variant, derivative or homologue thereof.

According to a fourth aspect of the present invention there is provided a promoter comprising the sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.

According to a fifth aspect of the present invention there is provided a construct capable of comprising or expressing the present invention.

According to a sixth aspect of the present invention there is provided a vector comprising or expressing the present invention.

According to a seventh aspect of the present invention there is provided a cell, tissue or organ comprising or expressing the present invention.

According to an eighth aspect of the present invention there is provided a transgenic starch producing organism comprising or expressing the present invention. According to a ninth aspect of the present invention there is provided a starch obtained from the present invention.

A key advantage of the present invention is that it provides a method for preparing modified starches that is not dependent on the need for post-harvest modification of starches. Thus the method of the present invention obviates the need for the use of hazardous chemicals that are normally used in the post-harvest modification of starches.

In addition, the present invention provides *inter alia* genetically modified plants which are capable of producing modified and/or novel and/or improved starches whose properties would satisfy various industrial requirements.

Thus, the present invention provides a method of preparing tailor-made starches in plants which could replace the post-harvest modified starches.

Also, the present invention provides a method that enables modified starches to be prepared by a method that can have a more beneficial effect on the environment than the known post-harvest modification methods which are dependent on the use of hazardous chemicals and large quantities of energy.

An other key advantage of the present invention is that it provides a method that may more reliably and/or more efficiently and/or more specifically affect enzymatic activity when compared to the known methods of affecting enzymatic activity. With regard to this advantage of the present invention it is to be noted that there is some degree of homology between coding regions of SBEs. However, there is little or no homology with the intron sequences of SBEs. Thus, sense intron expression provides a mechanism to affect selectively the expression of a particular SBE. This advantageous aspect could be used, for example, to reduce or eliminate a particular SBE enzyme and replace that enzyme with another enzyme which can be another branching enzyme or even a recombinant version of the affected enzyme or even a hybrid enzyme which could for example comprise part of a SBE enzyme from one source and at least a part of another SBE enzyme from another source. This particular feature of the present invention is

covered by the combination aspect of the present invention which is discussed in more detail later.

Thus the present invention provides a mechanism for selectively affecting SBE activity. This is in contrast to the prior art methods which are dependent on the use of for example antisense exon expression whereby it would not be possible to introduce new SBE activity without affecting that activity as well.

In the context of the present invention, class B SBE is synonymous with SBE I: class A SBE is synonymous with SBE II. Class A SBE is as defined in WO96/34968, incorporated herein by reference. Preferably, the antisense intron construct used comprises intron 1 of class A SBE, which is 2.0 kb in length and is located starting at residue 45 of the coding sequence of class A SBE. The boundaries of the intron may be calculated by searching for consensus intron boundary sequences, and are shown in attached figure 11. The sequence of the intron is set forth in SEQ. ID. No. 38. Class B SBE is substantially as defined in the sequences given herein and in PCT/EP96/03053.

Preferably with the first aspect of the present invention starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.

Preferably with the first or second aspect of the present invention the nucleotide sequence does not contain a sequence that is sense to an exon sequence.

Preferably with the fourth aspect of the present invention the promoter is in combination with a gene of interest ("GOI").

Preferably the enzymatic activity is reduced or eliminated.

Preferably the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

Preferably the nucleotide sequence codes, partially or completely, for two or more introns and wherein each intron is in a sense orientation.

Preferably the nucleotide sequence comprises at least 350 nucleotides (e.g. 350 bp), more preferably at least 500 nucleotides (e.g. 500 bp).

Preferably the nucleotide sequence comprises the sequence shown as SEQ. ID. No. 38, or a fragment thereof.

Preferably the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ. I.D. No. 14 or a variant, derivative or homologue thereof.

Preferably the transgenic starch producing organism is a plant.

A preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for a class A SBE intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

A more preferred aspect of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of

starch is changed; and wherein the nucleotide sequence comprises the sequence shown as SEQ. ID. No. 38, or fragments thereof.

The term "nucleotide" in relation to the present invention includes DNA and RNA. Preferably it means DNA, more preferably DNA prepared by use of recombinant DNA techniques.

The term "intron" is used in its normal sense as meaning a segment of nucleotides, usually DNA, that does not encode part or all of an expressed protein or enzyme.

The term "exon" is used in its normal sense as meaning a segment of nucleotides, usually DNA, encoding part or all of an expressed protein or enzyme.

Thus, the term "intron" refers to gene regions that are transcribed into RNA molecules, but which are spliced out of the RNA before the RNA is translated into a protein. In contrast, the term "exon" refers to gene regions that are transcribed into RNA and subsequently translated into proteins.

The terms "variant" or "homologue" or "fragment" in relation to the nucleotide sequence of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective nucleotide sequence providing the resultant nucleotide sequence can affect enzyme activity in a plant, or cell or tissue thereof, preferably wherein the resultant nucleotide sequence has at least the same effect as the sequence shown in SEQ. ID. No. 38. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant nucleotide sequence has the ability to affect enzymatic activity in accordance with the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

Likewise, the terms "variant" or "homologue" or "fragment" in relation to the promoter of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the respective promoter sequence providing the resultant promoter sequence allows expression of a GOI, preferably wherein the resultant promoter sequence has at least the same effect as SEQ.I.D. No. 14. In particular, the term "homologue" covers homology with respect to similarity of structure and/or similarity of function providing the resultant promoter sequence has the ability to allow for expression of a GOI, such as a nucleotide sequence according to the present invention. With respect to sequence homology (i.e. similarity), preferably there is more than 80% homology, more preferably at least 85% homology, more preferably at least 90% homology, even more preferably at least 95% homology, more preferably at least 98% homology. The above terms are also synonymous with allelic variations of the sequences.

The intron sequence of the present invention can be any one or all of the intron sequences of the present invention, including partial sequences thereof, provided that if partial sense sequences are used the partial sequences affect enzymatic activity. Suitable examples of partial sequences include sequences that are shorter than any one of the full sense sequences shown as SEQ. ID. No. 38 but which comprise nucleotides that are adjacent the respective exon or exons.

With regard to the second aspect of the present invention (i.e. specifically affecting SBE activity), the nucleotide sequences of the present invention may comprise one or more sense or antisense exon sequences of the class A or class B SBE gene (but not sense exon sequences naturally associated with the intron sequence), including complete or partial sequences thereof, providing the nucleotide sequences can affect SBE activity, preferably wherein the nucleotide sequences reduce or eliminate SBE activity. Preferably, the nucleotide sequence of the second aspect of the present invention does not comprise sense exon sequences.

The term "vector" includes an expression vector and a transformation vector. The term "expression vector" means a construct capable of *in vivo* or *in vitro* expression. The term "transformation vector" means a construct capable of being transferred from one species to another - such as from an *E.Coli* plasmid to a fungus or a plant cell, or from an *Agrobacterium* to a plant cell.

The term "construct" - which is synonymous with terms such as "conjugate", "cassette" and "hybrid" - in relation to the sense nucleotide sequence aspect of the present invention includes the nucleotide sequence according to the present invention directly or indirectly attached to a promoter. An example of an indirect attachment is the provision of a suitable spacer group such as an intron sequence, such as the *Sh1*-intron or the ADH intron, intermediate the promoter and the nucleotide sequence of the present invention. The same is true for the term "fused" in relation to the present invention which includes direct or indirect attachment. The terms do not cover the natural combination of the wild type SBE gene when associated with the wild type SBE gene promoter in their natural environment.

The construct may even contain or express a marker which allows for the selection of the genetic construct in, for example, a plant cell into which it has been transferred. Various markers exist which may be used in, for example, plants - such as mannose. Other examples of markers include those that provide for antibiotic resistance - e.g. resistance to G418, hygromycin, bleomycin, kanamycin and gentamycin.

The construct of the present invention preferably comprises a promoter. The term "promoter" is used in the normal sense of the art, e.g. an RNA polymerase binding site in the Jacob-Monod theory of gene expression. Examples of suitable promoters are those that can direct efficient expression of the nucleotide sequence of the present invention and/or in a specific type of cell. Some examples of tissue specific promoters are disclosed in WO 92/11375.

The promoter could additionally include conserved regions such as a Pribnow Box or a TATA box. The promoters may even contain other sequences to affect (such as to maintain, enhance, decrease) the levels of expression of the nucleotide sequence of the present invention. Suitable examples of such sequences include the *Sh1*-intron or an ADH intron. Other sequences include inducible elements - such as temperature, chemical, light or stress inducible elements. Also, suitable elements to enhance transcription or translation may be present. An example of the latter element is the TMV 5' leader sequence (see Sleat Gene 217 [1987] 217-225; and Dawson Plant Mol. Biol. 23 [1993] 97).

As mentioned, the construct and/or the vector of the present invention may include a transcriptional initiation region which may provide for regulated or constitutive expression. Any suitable promoter may be used for the transcriptional initiation region, such as a tissue specific promoter. In one aspect, preferably the promoter is the patatin promoter or the E35S promoter. In another aspect, preferably the promoter is the SBE promoter.

If, for example, the organism is a plant then the promoter can be one that affects expression of the nucleotide sequence in any one or more of seed, tuber, stem, sprout, root and leaf tissues, preferably tuber. By way of example, the promoter for the nucleotide sequence of the present invention can be the α-Amy 1 promoter (otherwise known as the Amy 1 promoter, the Amy 637 promoter or the α-Amy 637 promoter) as described in our co-pending UK patent application No. 9421292.5 filed 21 October 1994. Alternatively, the promoter for the nucleotide sequence of the present invention can be the α-Amy 3 promoter (otherwise known as the Amy 3 promoter, the Amy 351 promoter or the α-Amy 351 promoter) as described in our co-pending UK patent application No. 9421286.7 filed 21 October 1994.

The present invention also encompasses the use of a promoter to express a nucleotide sequence according to the present invention, wherein a part of the promoter is inactivated but wherein the promoter can still function as a promoter. Partial inactivation of a

promoter in some instances is advantageous. In particular, with the Amy 351 promoter mentioned earlier it is possible to inactivate a part of it so that the partially inactivated promoter expresses the nucleotide sequence of the present invention in a more specific manner such as in just one specific tissue type or organ. The term "inactivated" means partial inactivation in the sense that the expression pattern of the promoter is modified but wherein the partially inactivated promoter still functions as a promoter. However, as mentioned above, the modified promoter is capable of expressing a gene coding for the enzyme of the present invention in at least one (but not all) specific tissue of the original promoter. Examples of partial inactivation include altering the folding pattern of the promoter sequence, or binding species to parts of the nucleotide sequence, so that a part of the nucleotide sequence is not recognised by, for example, RNA polymerase. Another, and preferable, way of partially inactivating the promoter is to truncate it to form fragments thereof. Another way would be to mutate at least a part of the sequence so that the RNA polymerase can not bind to that part or another part. Another modification is to mutate the binding sites for regulatory proteins for example the CreA protein known from filamentous fungi to exert carbon catabolite repression, and thus abolish the catabolite repression of the native promoter.

The construct and/or the vector of the present invention may include a transcriptional termination region.

The nucleotide according to the present invention can be expressed in combination (but not necessarily at the same time) with an additional construct. Thus the present invention also provides a combination of constructs comprising a first construct comprising the nucleotide sequence according to the present invention operatively linked to a first promoter; and a second construct comprising a GOI operatively linked to a second promoter (which need not be the same as the first promoter). With this aspect of the present invention the combination of constructs may be present in the same vector, plasmid, cells, tissue, organ or organism. This aspect of the present invention also covers methods of expressing the same, preferably in specific cells or tissues, such as expression in just a specific cell or tissue, of an organism, typically a plant. With this aspect of the

present invention the second construct does not cover the natural combination of the gene coding for an enzyme ordinarily associated with the wild type gene promoter when they are both in their natural environment.

An example of a suitable combination would be a first construct comprising the nucleotide sequence of the present invention and a promoter, such as the promoter of the present invention, and a second construct comprising a promoter, such as the promoter of the present invention, and a GOI wherein the GOI codes for another starch branching enzyme either in sense or antisense orientation.

The above comments relating to the term "construct" for the sense nucleotide aspect of the present invention are equally applicable to the term "construct" for the promoter aspect of the present invention. In this regard, the term includes the promoter according to the present invention directly or indirectly attached to a GOI.

The term "GOI" with reference to the promoter aspect of the present invention or the combination aspect of the present invention means any gene of interest, which need not necessarily code for a protein or an enzyme - as is explained later. A GOI can be any nucleotide sequence that is either foreign or natural to the organism in question, for example a plant.

Typical examples of a GOI include genes encoding for other proteins or enzymes that modify metabolic and catabolic processes. The GOI may code for an agent for introducing or increasing pathogen resistance.

The GOI may even be an antisense construct for modifying the expression of natural transcripts present in the relevant tissues. An example of such a GOI is the nucleotide sequence according to the present invention.

The GOI may even code for a protein that is non-natural to the host organism - e.g. a plant. The GOI may code for a compound that is of benefit to animals or humans. For example, the GOI could code for a pharmaceutically active protein or enzyme such as any one of the therapeutic compounds insulin, interferon, human serum albumin, human growth factor and blood clotting factors. The GOI may even code for a protein giving additional nutritional value to a food or feed or crop. Typical examples include plant proteins that can inhibit the formation of anti-nutritive factors and plant proteins that have a more desirable amino acid composition (e.g. a higher lysine content than a non-transgenic plant). The GOI may even code for an enzyme that can be used in food processing such as xylanases and α-galactosidase. The GOI can be a gene encoding for any one of a pest toxin, an antisense transcript such as that for α-amylase, a protease or a glucanase. Alternatively, the GOI can be a nucleotide sequence according to the present invention.

The GOI can be the nucleotide sequence coding for the arabinofuranosidase enzyme which is the subject of our co-pending UK patent application 9505479.7. The GOI can be the nucleotide sequence coding for the glucanase enzyme which is the subject of our co-pending UK patent application 9505475.5. The GOI can be the nucleotide sequence coding for the α-amylase enzyme which is the subject of our co-pending UK patent application 9413439.2. The GOI can be the nucleotide sequence coding for the α-amylase enzyme which is the subject of our co-pending UK patent application 9421290.9. The GOI can be any of the nucleotide sequences coding for the α-glucan lyase enzyme which are described in our co-pending PCT patent application PCT/EP94/03397.

In one aspect the GOI can even be a nucleotide sequence according to the present invention but when operatively linked to a different promoter.

The GOI could include a sequence that codes for one or more of a xylanase, an arabinase, an acetyl esterase, a rhamnogalacturonase, a glucanase, a pectinase, a branching enzyme or another carbohydrate modifying enzyme or proteinase. Alternatively, the GOI may be a sequence that is antisense to any of those sequences.

As mentioned above, the present invention provides a mechanism for selectively affecting a particular enzymatic activity.

In an important application of the present invention it is now possible to reduce or eliminate expression of a genomic nucleotide sequence coding for a genomic protein or enzyme by expressing a sense intron construct for that particular genomic protein or enzyme and (e.g. at the same time) expressing a recombinant version of that enzyme or protein - in other words the GOI is a recombinant nucleotide sequence coding for the genomic enzyme or protein. This application allows expression of desired recombinant enzymes and proteins in the absence of (or reduced levels of) respective genomic enzymes and proteins. Thus the desired recombinant enzymes and proteins can be easily separated and purified from the host organism. This particular aspect of the present invention is very advantageous over the prior art methods which, for example, rely on the use of anti-sense exon expression which methods also affect expression of the recombinant enzyme.

Thus, a further aspect of the present invention relates to a method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence wherein the further nucleotide sequence codes, partially or completely, for an intron in a sense-orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron. Additional aspects cover the combination of those nucleotide sequences including their incorporation in constructs, vectors, cells, tissues and transgenic organisms.

Therefore the present invention also relates to a combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to an intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding the enzyme

corresponding to the recombinant enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

The GOI may even code for one or more introns but in an antisense orientation, such as any one or more of the antisense intron sequences presented in the attached sequence listings. For example, the present invention also covers the expression of for example a sense intron (e.g. SEQ.I.D.No. 38) in combination with for example an antisense intron which preferably is not complementary to the sense intron sequence (e.g. SEQ.I.D.No. 16).

The terms "cell", "tissue" and "organ" include cell, tissue and organ *per se* and when within an organism.

The term "organism" in relation to the present invention includes any organism that could comprise the nucleotide sequence according to the present invention and/or wherein the nucleotide sequence according to the present invention can be expressed when present in the organism. Preferably the organism is a starch producing organism such as any one of a plant, algae, fungi, yeast and bacteria, as well as cell lines thereof. Preferably the organism is a plant.

The term "starch producing organism" includes any organism that can biosynthesise starch. Preferably, the starch producing organism is a plant.

The term "plant" as used herein includes any suitable angiosperm, gymnosperm, monocotyledon and dicotyledon. Typical examples of suitable plants include vegetables such as potatoes; cereals such as wheat, maize, and barley; fruit; trees; flowers; and other plant crops. Preferably, the term means "potato".

The term "transgenic organism" in relation to the present invention includes any organism that comprises the nucleotide sequence according to the present invention and/or products

obtained therefrom, and/or wherein the nucleotide sequence according to the present invention can be expressed within the organism. Preferably the nucleotide sequence of the present invention is incorporated in the genome of the organism. Preferably the transgenic organism is a plant, more preferably a potato.

To prepare the host organism one can use prokaryotic or eukaryotic organisms. Examples of suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Teachings on the transformation of prokaryotic hosts is well documented in the art, for example see Sambrook *et al* (Sambrook *et al*. in Molecular Cloning: A Laboratory Manual, 2nd edition, 1989, Cold Spring Harbor Laboratory Press).

Even though the enzyme according to the present invention and the nucleotide sequence coding for same are not disclosed in EP-B-0470145 and CA-A-2006454, those two documents do provide some useful background commentary on the types of techniques that may be employed to prepare transgenic plants according to the present invention. Some of these background teachings are now included in the following commentary.

The basic principle in the construction of genetically modified plants is to insert genetic information in the plant genome so as to obtain a stable maintenance of the inserted genetic material.

Several techniques exist for inserting the genetic information, the two main principles being direct introduction of the genetic information and introduction of the genetic information by use of a vector system. A review of the general techniques may be found in articles by Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27).

Thus, in one aspect, the present invention relates to a vector system which carries a nucleotide sequence or construct according to the present invention and which is capable of introducing the nucleotide sequence or construct into the genome of an organism, such as a plant.

The vector system may comprise one vector, but it can comprise two vectors. In the case of two vectors, the vector system is normally referred to as a binary vector system. Binary vector systems are described in further detail in Gynheung An *et al.* (1980), Binary Vectors, *Plant Molecular Biology Manual A3*, 1-19.

One extensively employed system for transformation of plant cells with a given promoter or nucleotide sequence or construct is based on the use of a Ti plasmid from *Agrobacterium tumefaciens* or a Ri plasmid from *Agrobacterium rhizogenes* An *et al.* (1986), *Plant Physiol.* 81, 301-305 and Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists,* eds.: D.S. Ingrams and J.P. Helgeson, 203-208. Several different Ti and Ri plasmids have been constructed which are suitable for the construction of the plant or plant cell constructs described above. A non-limiting example of such a Ti plasmid is pGV3850.

The nucleotide sequence or construct of the present invention should preferably be inserted into the Ti-plasmid between the terminal sequences of the T-DNA or adjacent a T-DNA sequence so as to avoid disruption of the sequences immediately surrounding the T-DNA borders, as at least one of these regions appears to be essential for insertion of modified T-DNA into the plant genome.

As will be understood from the above explanation, if the organism is a plant the vector system of the present invention is preferably one which contains the sequences necessary to infect the plant (e.g. the *vir* region) and at least one border part of a T-DNA sequence, the border part being located on the same vector as the genetic construct.

Furthermore, the vector system is preferably an *Agrobacterium tumefaciens* Ti-plasmid or an *Agrobacterium rhizogenes* Ri-plasmid or a derivative thereof. As these plasmids are well-known and widely employed in the construction of transgenic plants, many vector systems exist which are based on these plasmids or derivatives thereof.

In the construction of a transgenic plant the nucleotide sequence or construct of the present invention may be first constructed in a microorganism in which the vector can replicate and which is easy to manipulate before insertion into the plant. An example of a useful microorganism is *E. coli*, but other microorganisms having the above properties may be used. When a vector of a vector system as defined above has been constructed in *E. coli*, it is transferred, if necessary, into a suitable *Agrobacterium* strain, e.g. *Agrobacterium tumefaciens*. The Ti-plasmid harbouring the nucleotide sequence or construct of the present invention is thus preferably transferred into a suitable *Agrobacterium* strain, e.g. *A. tumefaciens*, so as to obtain an *Agrobacterium* cell harbouring the promoter or nucleotide sequence or construct of the present invention, which DNA is subsequently transferred into the plant cell to be modified.

If, for example, for the transformation the Ti- or Ri-plasmid of the plant cells is used, at least the right boundary and often however the right and the left boundary of the Ti- and Ri-plasmid T-DNA, as flanking areas of the introduced genes, can be connected. The use of T-DNA for the transformation of plant cells has been intensively studied and is described in EP-A-120516; Hoekema, in: The Binary Plant Vector System Offset-drukkerij Kanters B.B., Alblasserdam, 1985, Chapter V; Fraley, *et al.*, Crit. Rev. Plant Sci., 4:1-46; and An *et al.*, EMBO J. (1985) 4:277-284.

Direct infection of plant tissues by *Agrobacterium* is a simple technique which has been widely employed and which is described in Butcher D.N. *et al.* (1980), *Tissue Culture Methods for Plant Pathologists,* eds.: D.S. Ingrams and J.P. Helgeson, 203-208. For further teachings on this topic see Potrykus (Annu Rev Plant Physiol Plant Mol Biol [1991] 42:205-225) and Christou (Agro-Food-Industry Hi-Tech March/April 1994 17-27). With this technique, infection of a plant may be performed in or on a certain part or tissue of the plant, i.e. on a part of a leaf, a root, a stem or another part of the plant.

Typically, with direct infection of plant tissues by *Agrobacterium* carrying the GOI (such as the nucleotide sequence according to the present invention) and, optionally, a promoter, a plant to be infected is wounded, e.g. by cutting the plant with a razor blade

or puncturing the plant with a needle or rubbing the plant with an abrasive. The wound is then inoculated with the *Agrobacterium*. The inoculated plant or plant part is then grown on a suitable culture medium and allowed to develop into mature plants.

When plant cells are constructed, these cells may be grown and maintained in accordance with well-known tissue culturing methods such as by culturing the cells in a suitable culture medium supplied with the necessary growth factors such as amino acids, plant hormones, vitamins, etc.

Regeneration of the transformed cells into genetically modified plants may be accomplished using known methods for the regeneration of plants from cell or tissue cultures, for example by selecting transformed shoots using an antibiotic and by subculturing the shoots on a medium containing the appropriate nutrients, plant hormones, etc.

Further teachings on plant transformation may be found in EP-A-0449375.

As reported in CA-A-2006454, a large amount of cloning vectors are available which contain a replication system in *E. coli* and a marker which allows a selection of the transformed cells. The vectors contain for example pBR 322, pUC series, M13 mp series, pACYC 184 etc. In this way, the nucleotide or construct of the present invention can be introduced into a suitable restriction position in the vector. The contained plasmid is then used for the transformation in *E.coli*. The *E.coli* cells are cultivated in a suitable nutrient medium and then harvested and lysed. The plasmid is then recovered. As a method of analysis there is generally used sequence analysis, restriction analysis, electrophoresis and further biochemical-molecular biological methods. After each manipulation, the used DNA sequence can be restricted and connected with the next DNA sequence. Each sequence can be cloned in the same or different plasmid.

After the introduction of the nucleotide sequence or construct according to the present invention in the plants the presence and/or insertion of further DNA sequences may be

necessary - such as to create combination systems as outlined above (e.g. an organism comprising a combination of constructs).

The above commentary for the transformation of prokaryotic organisms and plants with the nucleotide sequence of the present invention is equally applicable for the transformation of those organisms with the promoter of the present invention.

In summation, the present invention relates to affecting enzyme activity by expressing sense intron sequences.

Also, the present invention relates to a promoter useful for the expression of those sense intron sequences.

The following samples have been deposited in accordance with the Budapest Treaty at the recognised depositary The National Collections of Industrial and Marine Bacteria Limited (NCIMB) at 23 St Machar Drive, Aberdeen, Scotland, AB2 1RY, United Kingdom, on 13 July 1995:

NCIMB 40754 (which refers to pBEA 11 as described herein);

NCIMB 40751 (which refers to λ-SBE 3.2 as described herein), and

NCIMB 40752 (which refers to λ-SBE 3.4 as described herein).

A highly preferred embodiment of the present invention therefore relates to a method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron in a sense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of

starch is changed; and wherein the intron nucleotide sequence is the sequence of intron 1 of class A SBE as set forth in SEQ. ID. No. 38, or any other intron of class A SBE, including fragments thereof, and including combinations of class A sense intron sequences and class B sense or antisense intron sequences. The sequence of introns of class A SBE other than intron 1 may be obtained by sequencing of, for example, potato class A SBE genomic DNA, isolatable by hybridisation screening of a genomic DNA library with class A SBE cDNA obtainable according to WO96/34968 according to methods well known in the art and set forth, for example, in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1989.

The present invention will now be described only by way of example, in which reference is made to the following attached Figures:

Figure 1, which is a schematic representation of the biosynthesis of amylose and amylopectin;

Figure 2, which is a diagrammatic representation of the α-1-4-links and the α-1-6 links of amylopectin;

Figure 3, which is a diagrammatic representation of the exon-intron structure of a genomic SBE clone;

Figure 4, which is a plasmid map of pPATA1, which is 3936 bp in size;

Figure 5, which is a plasmid map of pABE7, which is 5106 bp in size;

Figure 6, which is a plasmid map of pVictorIV Man, which is 7080 bp in size;

Figure 7, which is a plasmid map of pBEA11, which is 9.54 kb in size;

Figure 8, which shows the full genomic nucleotide sequence for SBE including the promoter, exons and introns;

Figure 9, which is a plasmid map of pVictor5a, which is 9.12 kb in size;

Figure 10, which is a plasmid map of pBEP2, which is 10.32 kb in size;

Figure 11, which shows the positioning of intron 1 in the class A and class B SBE genes;

Figure 12, which shows the sequence of intron 1 of the potato class A SBE;

Figure 13, which shows pSS15; and

Figure 14, which shows pSS16.

Figures 1 and 2 were referred to above in the introductory description concerning starch in general. As mentioned, Figure 3 is a diagrammatic representation of the exon-intron structure of a genomic SBE clone, the sequence of which is shown in Figure 8. This clone, which has about 11.5 k base pairs, comprises 14 exons and 13 introns. The introns are numbered in increasing order from the 5' end to the 3' end and correspond to SEQ.I.D.No.s 1-13, respectively. Their respective antisense intron sequences are shown as SEQ.I.D.No.s 15-27.

In more detail, Figures 3 and 8 present information on the 11468 base pairs of a potato SBE gene. The 5' region from nucleotides 1 to 2082 contain the promoter region of the SBE gene. A TATA box candidate at nucleotide 2048 to 2051 is boxed. The homology between a potato SBE cDNA clone (Poulsen & Kreiberg (1993) Plant Physiol 102: 1053-1054) and the exon DNAs begin at 2083 bp and end at 9666 bp. The homology between the cDNA and the exon DNA is indicated by nucleotides in upper case letters, while the translated amino acid sequences are shown in the single letter code below the exon DNA. Intron sequences are indicated by lower case letters.

Figure 7 is a plasmid map of pBEA7, which is 9.54 k base pairs in size. Plasmid pBEA 11 comprises the first intron sequence of the potato SBE gene. This first intron sequence, which has 1177 base pairs, is shown in Figure 3 and lies between the first exon and the second exon.

These experiments and aspects of the present invention are now discussed in more detail.

EXPERIMENTAL PROTOCOL

ISOLATION, SUBCLONING IN PLASMIDS, AND SEQUENCING OF GENOMIC SBE CLONES

Various clones containing the potato SBE gene are isolated from a Desiree potato genomic library (Clontech Laboratories Inc., Palo Alto CA, USA) using radioactively labelled potato SBE cDNA (Poulsen & Kreiberg (1993) Plant Physiol. 102:1053-1054) as probe. The fragments of the isolated λ-phages containing SBE DNA (λSBE 3.2 - NCIMB 40751 - and λSBE-3.4 - NCIMB 40752) are identified by Southern analysis and then subcloned into pBluescript II vectors (Clontech Laboratories Inc., Palo Alto CA, USA). λSBE 3.2 contains a 15 kb potato DNA insert and λSBE-3.4 contains a 13 kb potato DNA insert. The resultant plasmids are called pGB3, pGB11, pGB15, pGB16 and pGB25 (see discussion below). The respective inserts are then sequenced using the Pharmacia Autoread Sequencing Kit (Pharmacia, Uppsala) and a A.L.F. DNA sequencer (Pharmacia, Uppsala).

In total, a stretch of 11.5 kb of the SBE gene is sequenced. The sequence is deduced from the above-mentioned plasmids, wherein: pGB25 contains the sequences from 1 bp to 836 bp, pGB15 contains the sequences from 735 bp to 2580 bp, pGB16 contains the sequences from 2580 bp to 5093 bp, pGB11 contains the sequences from 3348 bp to 7975 bp, and pGB3 contains the sequences from 7533 bp to 11468 bp.

In more detail, pGB3 is constructed by insertion of a 4 kb *EcoR*I fragment isolated from λSBE 3.2 into the *EcoR*I site of pBluescript II SK (+). pGB11 is constructed by

insertion of a 4.7 kb *Xho*I fragment isolated from λSBE 3.4 into the *Xho*I site of pBluescript II SK (+). pGB15 is constructed by insertion of a 1.7 kb *Spe*I fragment isolated from λSBE 3.4 into the *Spe*I site of pBluescript II SK (+). pGB16 is constructed by insertion of a 2.5 kb *Spe*I fragment isolated from λSBE 3.4 into the *Spe*I site of pBluescript II SK (+). For the construction of pGB25 a PCR fragment is produced with the primers

5' GGA ATT CCA GTC GCA GTC TAC ATT AC 3'

(SEQ. ID. No. 30)

and

5' CGG GAT CCA GAG GCA TTA AGA TTT CTG G 3'

(SEQ. ID. No. 31)

and λSBE 3.4 as a template.

The PCR fragment is digested with *BamH*I and *Eco*RI, and inserted in pBluescript II SK (+) digested with the same restriction enzymes.

CONSTRUCTION OF PLASMID pBEA11

The SBE intron 1 is amplified by PCR using the oligonucleotides

5' CGG GAT CCA AAG AAA TTC TCG AGG TTA CAT GG 3'

(SEQ. ID. No. 32)

and

5' CGG GAT CCG GGG TAA TTT TTA CTA ATT TCA TG 3'

(SEQ. ID. No. 33)

and the λSBE 3.4 phage containing the SBE gene as template.

The PCR product is digested with *BamH*I and inserted in a sense orientation in the *BamH*I site of plasmid pPATA1 (described in WO 94/24292) between the patatin promoter and the 35S terminator. This construction, pABE7, is digested with *Kpn*I, and the 2.4 kb "patatin promoter-SBE intron 1- 35S terminator" *Kpn*I fragment is isolated and inserted in the *Kpn*I site of the plant transformation vector pVictorIV Man yielding plasmid pBEA11.

CONSTRUCTION OF PLASMID pSS15.

The 2122 bp intron 1 sequence of the potato SBEII gene (see SEQ. ID. No. 38) is amplified by PCR from a genomic SBEII subclone using the primers 5' - CGG GAT CCC GTA TGT CTC ACT GTG TTT GTG GC - 3' (SEQ. ID. No. 34) and 5' - CGG GAT CCC CCT ACA TAC ATA TAT CAG ATT AG - 3' (SEQ. ID. No. 35). The PCR product is digested with BamHI and inserted in sense orientation after a patatin promoter in the BamHI site of a plant transformation vector in which the NPTII gene is used as selectable marker (see figure 13).

CONSTRUCTION OF PLASMID pSS16.

The 2122 bp intron 1 sequence of the potato SBEII gene (SEQ. ID. No. 38) is amplified by PCR from a genomic SBEII subclone using the primers 5' - CGG GAT CCC GTA TGT CTC ACT GTG TTT GTG GC - 3' (SEQ. ID. No. 34) and 5' - CGG GAT CCC CCT ACA TAC ATA TAT CAG ATT AG - 3' (SEQ. ID. No. 35). The PCR product is digested with BamHI and inserted in sense orientation after a patatin promoter in the BamHI site of a plant transformation vector in which the *manA* gene is used as selectable marker (see figure 14).

PRODUCTION OF TRANSGENIC POTATO PLANTS

Axenic stock cultures

Shoot cultures of *Solanum tuberosum* 'Bintje' and 'Dianella' are maintained on a substrate (LS) of a formula according to Linsmaier, E.U. and Skoog, F. (1965), Physiol. Plant. 18: 100-127, in addition containing 2 μM silver thiosulphate at 25°C and 16 h light/8 h dark.

The cultures are subcultured after approximately 40 days. Leaves are then cut off the shoots and cut into nodal segments (approximately 0.8 cm) each containing one node.

Inoculation of potato tissues

Shoots from approximately 40 days old shoot cultures (height approximately 5-6 cms) are cut into internodal segments (approximately 0.8 cm). The segments are placed into liquid LS-substrate containing the transformed *Agrobacterium tumefaciens* containing the binary vector of interest. The *Agrobacterium* are grown overnight in YMB-substrate

(di-potassium hydrogen phosphate, trihydrate (0.66 g/l); magnesium sulphate, heptahydrate (0.20 g/l); sodium chloride (0.10 g/l); mannitol (10.0 g/l); and yeast extract (0.40 g/l)) containing appropriate antibiotics (corresponding to the resistance gene of the *Agrobacterium* strain) to an optical density at 660 nm (OD-660) of approximately 0.8, centrifuged and resuspended in the LS-substrate to an OD-660 of 0.5.

The segments are left in the suspension of *Agrobacterium* for 30 minutes and then the excess of bacteria are removed by blotting the segments on sterile filter paper.

Co-cultivation

The shoot segments are co-cultured with bacteria for 48 hours directly on LS-substrate containing agar (8.0 g/l), 2,4-dichlorophenoxyacetic acid (2.0 mg/l) and trans-zeatin (0.5 mg/l). The substrate and also the explants are covered with sterile filter papers, and the petri dishes are placed at 25°C and 16 h light/ 8 dark.

"Washing" procedure

After the 48 h on the co-cultivation substrate the segments are transferred to containers containing liquid LS-substrate containing 800 mg/l carbenicillin. The containers are gently shaken and by this procedure the major part of the *Agrobacterium* is either washed off the segments and/or killed.

Selection

After the washing procedure the segments are transferred to plates containing the LS-substrate, agar (8 g/l), trans-zeatin (1-5 mg/l), gibberellic acid (0.1 mg/l), carbenicillin (800 mg/l), and kanamycin sulphate (50-100 mg/l) or phosphinotricin (1-5 mg/l) or mannose (5 g/l) depending on the vector construction used. The segments are sub-cultured to fresh substrate each 3-4 weeks. In 3 to 4 weeks, shoots develop from the segments and the formation of new shoots continued for 3-4 months.

Rooting of regenerated shoots

The regenerated shoots are transferred to rooting substrate composed of LS-substrate, agar (8 g/l) and carbenicillin (800 mg/l). The transgenic genotype of the

regenerated shoot are verified by testing the rooting ability on the above mentioned substrates containing kanamycin sulphate (200 mg/l), by performing NPTII assays (Radke, S. E. et al, Theor. Appl. Genet. (1988), 75: 685-694) or by performing PCR analysis according to Wang *et al* (1993, NAR 21 pp 4153-4154). Plants which are not positive in any of these assays are discarded or used as controls. Alternatively, the transgenic plants could be verified by performing a GUS assay on the co-introduced β-glucuronidase gene according to Hodal, L. *et al.* (Pl. Sci. (1992), 87: 115-122).

Transfer to soil

The newly rooted plants (height approx. 2-3 cms) are transplanted from rooting substrate to soil and placed in a growth chamber (21°C, 16 hour light 200-400uE/m^2/sec). When the plants are well established they are transferred to the greenhouse, where they are grown until tubers had developed and the upper part of the plants are senescing.

Harvesting

The potatoes are harvested after about 3 months and then analysed.

BRANCHING ENZYME ANALYSIS

The SBE expression in the transgenic potato lines are measured using the SBE assays described by Blennow and Johansson (Phytochemistry (1991) 30:437-444) and by standard Western procedures using antibodies directed against class A and class B potato SBE.

STARCH ANALYSIS

Starch is isolated from potato tubers and analysed for the amylose:amylopectin ratio (Hovenkamp-Hermelink et al. (1988) Potato Research 31:241-246). In addition, the chain length distribution of amylopectin is determined by analysis of isoamylase digested starch on a Dionex HPAEC. The number of reducing ends in isoamylase digested starch is determined by the method described by N. Nelson (1944) J. Biol.Chem. 153:375-380.

The results revealed that there is a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch SBE in the transgenic plants.

CONSTRUCTION OF SBE PROMOTER CONSTRUCT

An SBE promoter fragment is amplified from λ-SBE 3.4 using primers:

5´ CCA TCG ATA CTT TAA GTG ATT TGA TGG C 3'

(SEQ. ID. No. 36)

and

5' CGG GAT CCT GTT CTG ATT CTT GAT TTC C 3'.

(SEQ. ID. No. 37)

The PCR product is digested with *Cla*I and *BamH*I. The resultant 1.2 kb fragment is then inserted in pVictor5a (see Figure 9) linearised with *Cla*I and *Bgl*II yielding pBEP2 (see Figure 10).

STARCH BRANCHING ENZYME MEASUREMENTS OF POTATO TUBERS

Potatoes from potato plants transformed with pBEA11 are cut in small pieces and homogenised in extraction buffer (50 mM Tris-HCl pH 7.5, Sodium-dithionite (0.1 g/l), and 2 mM DTT) using a Ultra-Turax homogenizer; 1 g of Dowex xl. is added pr. 10 g of tuber. The crude homogenate is filtered through a miracloth filter and centrifuged at 4°C for 10 minutes at 24.700 g. The supernatant is used for starch branching enzyme-assays. The starch branching enzyme assays are carried out at 25 °C in a volume of 400 µl composed of 0.1 M Na citrate buffer pH 7.0, 0.75 mg/ml amylose, 5 mg/ml bovine serum albumin and the potato extract. At 0, 15 30 and 60 minutes aliqouts of 50 µl are removed from the reaction into 20 µl 3 N HCl. 1 ml of iodine solution is added and the decrease in absorbance at 620 nm is measured with an ELISA spectrophotometer.

The starch branching enzyme (SBE) levels in tuber extracts are measured from 24 transgenic Dianella potato plants transformed with plasmid pBEA11, pSS15 and pSS16.

The results show that the BEA11, SS15 and SS16 transgenic lines produce tubers which have class B and class A SBE levels, respectively, that are only 10 % to 15 % of the SBE levels found in non transformed Dianella plants.

In a further experiment, plasmids pSS15 and pBEA11 are cotransfected into potato plants, as described above. In the cotransfectants, when analysed as set forth above, simultaneous reduction of class A and class B SBE levels are observed.

SUMMATION

The above-mentioned examples relate to the isolation and sequencing of a gene for potato SBE. The examples further demonstrate that it is possible to prepare SBE intron constructs. These SBE intron constructs can be introduced into plants, such as potato plants. After introduction, a reduction in the level of synthesis of SBE and/or the level of activity of SBE and/or the composition of starch in plants can be achieved.

Without wishing to be bound by theory it is believed that the expressed sense intron nucleotide sequence according to the present invention affects enzymatic activity *via* co-suppression and/or trans-activation. Reviews of these mechanisms has been published by Finnegan and McElroy (1994 Biotechnology 12 pp 883 - 887) and Matzke and Matzke (1995 TIG 11 No. 1 pp 1 - 3). By these mechanisms, it is believed that the sense introns of the present invention reduce the level of plant enzyme activity (in particular SBE activity), which in turn for SBE activity is believed to influence the amylose:amylopectin ratio and thus the branching pattern of amylopectin.

Thus, the present invention provides a method wherein it is possible to manipulate the starch composition in plants, or tissues or cells thereof, such as potato tubers, by reducing the level of SBE activity by using sense intron sequences.

The simultaneous reduction or elimination of class A and class B SBE sequences from the doubly transformed potato plants, moreover, offers the possibility to transform such plants with different SBE genes at will, thus allowing the manipulation of branching in starch according to the desired result.

In summation the present invention therefore relates to the surprising use of SBE class A sense intron sequences in a method to affect class A SBE activity in plants.

Other modifications of the present invention will be apparent to those skilled in the art without departing from the scope of the present invention.

The following pages present a number of sequence listings which have been consecutively numbered from SEQ.I.D. No. 1 - SEQ.I.D. No. 38. In brief, SEQ.I.D. No. 1 - SEQ.I.D. No. 13 represent sense intron sequences (genomic DNA); SEQ.I.D.

No. 14 represents the SBE promoter sequence (genomic sequence); SEQ.I.D. No. 15 - SEQ.I.D. No. 27 represent antisense intron sequences; and SEQ. I.D. No. 28 represents the sequence complementary to the SBE promoter sequence - i.e. the SBE promoter sequence in antisense orientation. The full genomic nucleotide sequence for SBE including the promoter, exons and introns is shown as SEQ. I.D. No. 29 (see Figures 3 and 8 which highlight particular gene features). SEQ. ID. No. 30 to 37 show primers used in the methods set forth above. SEQ. ID. No. 38 represents the nucleotide sequence of intron 1 of the class A potato SBE gene.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: DANISCO A/S
(B) STREET: LANGEBROGADE 1
(C) CITY: COPENHAGEN K
(E) COUNTRY: DENMARK
(F) POSTAL CODE (ZIP): DK-1001

(ii) TITLE OF INVENTION: INHIBITION OF GENE EXPRESSION

(iii) NUMBER OF SEQUENCES: 38

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1165 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTAATTTTTA CTAATTTCAT GTTAATTTCA ATTATTTTTA GCCTTTGCAT TTCATTTTCC 60

AATATATCTG GATCATCTCC TTAGTTTTTT ATTTTATTTT TTATAATATC AAATATGGAA 120

GAAAAATGAC ACTTGTAGAG CCATATGTAA GTATCATGTG ACAAATTTGC AAGGTGGTTG 180

AGTGTATAAA ATTCAAAAAT TGAGAGATGG AGGGGGGGTG GGGGAAGACA ATATTTAGAA 240

AGAGTGTTCT AGGAGGTTAT GGAGGACACG GATGAGGGGT AGAAGGTTAG TTAGGTATTT 300

GAGTGTTGTC TGGCTTATCC TTTCATACTA GTAGTCGTGG AATTATTTGG GTAGTTTCTT 360

GTTTTGTTAT TTGATCTTTG TTATTCTATT TTCTGTTTCT TGTACTTCGA TTATTGTATT 420

ATATATCTTG TCGTAGTTAT TGTTCCTCGG TAAGAATGCT CTAGCATGCT TCCTTTAGTG 480

TTTTATCATG CCTTCTTTAT ATTCGCGTTG CTTTGAAATG CTTTTACTTT AGCCGAGGGT 540

CTATTAGAAA CAATCTCTCT ATCTCGTAAG GTAGGGGTAA AGTCCTCACC ACACTCCACT 600

TGTGGGATTA CATTGTGTTT GTTGTTGTAA ATCAATTATG TATACATAAT AAGTGGATTT 660

TTTACAACAC AAATACATGG TCAAGGGCAA AGTTCTGAAC ACATAAAGGG TTCATTATAT 720

GTCCAGGGAT ATGATAAAAA TTGTTTCTTT GTGAAAGTTA TATAAGATTT GTTATGGCTT 780

TTGCTGGAAA CATAATAAGT TATAATGCTG AGATAGCTAC TGAAGTTTGT TTTTTCTAGC 840

CTTTTAAATG TACCAATAAT AGATTCCGTA TCGAACGAGT ATGTTTTGAT TACCTGGTCA 900

TGATGTTTCT ATTTTTTACA TTTTTTTGGT GTTGAACTGC AATTGAAAAT GTTGTATCCT 960

ATGAGACGGA TAGTTGAGAA TGTGTTCTTT GTATGGACCT TGAGAAGCTC AAACGCTACT 1020

CCAATAATTT CTATGAATTC AAATTCAGTT TATGGCTACC AGTCAGTCCA GAAATTAGGA 1080

TATGCTGCAT ATACTTGTTC AATTATACTG TAAAATTTCT TAAGTTCTCA AGATATCCAT 1140

GTAACCTCGA GAATTTCTTT GACAG 1165

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 317 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GTATGTTTGA TAATTTATAT GGTTGCATGG ATAGTATATA AATAGTTGGA AAACTTCTGG 60

ACTGGTGCTC ATGGCATATT TGATCTGTGC ACCGTGTGGA GATGTCAAAC ATGTGTTACT 120

TCGTTCCGCC AATTTATAAT ACCTTAACTT GGGAAAGACA GCTCTTTACT CCTGTGGGCA 180

TTTGTTATTT GAATTACAAT CTTTATGAGC ATGGTGTTTT CACATTATCA ACTTCTTTCA 240

TGTGGTATAT AACAGTTTTT AGCTCCGTTA ATACCTTTCT TCTTTTTGAT ATAAACTAAC 300

TGTGGTGCAT TGCTTGC 317

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 504 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GTAACAGCCA AAAGTTGTGC TTTAGGCAGT TGACCTTAT TTTGGAAGAT GAATTGTTTA 60

TACCTACTTT GACTTTGCTA GAGAATTTTG CATACCGGGG AGTAAGTAGT GGCTCCATTT 120

AGGTGGCACC TGGCCATTTT TTTGATCTTT TAAAAAGCTG TTTGATTGGG TCTTCAAAAA 180

AGTAGACAAG GTTTTTGGAG AAGTGACACA CCCCCGGAGT GTCAGTGGCA AAGCAAAGAT 240

TTTCACTAAG GAGATTCAAA ATATAAAAAA AGTATAGACA TAAAGAAGCT GAGGGGATTC 300

AACATGTACT ATACAAGCAT CAAATATAGT CTTAAAGCAA TTTTGTAGAA ATAAAGAAAG 360

TCTTCCTTCT GTTGCTTCAC AATTTCCTTC TATTATCATG AGTTACTCTT TCTGTTCGAA 420

ATAGCTTCCT TAATATTAAA TTCATGATAC TTTTGTTGAG ATTTAGCAGT TTTTTCTTGT 480

GTAAACTGCT CTCTTTTTTT GCAG 504

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 146 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTAGGTCCTC GTCTACTACA AAATAGTAGT TTCCATCATC ATAACAGATT TTCCTATTAA 60

AGCATGATGT TGCAGCATCA TTGGCTTTCT TACATGTTCT AATTGCTATT AAGGTTATGC 120

TTCTAATTAA CTCATCCACA ATGCAG 146

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 218 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GTTTTGTTAT TCATACCTTG AAGCTGAATT TTGAACACCA TCATCACAGG CATTTCGATT 60

CATGTTCTTA CTAGTCTTGT TATGTAAGAC ATTTTGAAAT GCAAAAGTTA AAATAATTGT 120

GTCTTTACTA ATTTGGACTT GATCCCATAC TCTTTCCCTT AACAAAATGA GTCAATTCTA 180

TAAGTGCTTG AGAACTTACT ACTTCAGCAA TTAAACAG 218

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GTATTTTAAA TTTATTTCTA CAACTAAATA ATTCTCAGAA CAATTGTTAG ATAGAATCCA 60

AATATATACG TCCTGAAAGT ATAAAAGTAC TTATTTTCGC CATGGGCCTT CAGAATATTG 120

GTAGCCGCTG AATATCATGA TAAGTTATTT ATCCAGTGAC ATTTTTATGT TCACTCCTAT 180

TATGTCTGCT GGATACAG 198

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 208 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GTTTGTCTGT TTCTATTGCA TTTTAAGGTT CATATAGGTT AGCCACGGAA AATCTCACTC 60

TTTGTGAGGT AACCAGGGTT CTGATGGATT ATTCAATTTT CTCGTTTATC ATTTGTTTAT 120

TCTTTTCATG CATTGTGTTT CTTTTTCAAT ATCCCTCTTA TTTGGAGGTA ATTTTTCTCA 180

TCTATTCACT TTTAGCTTCT AACCACAG 208

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 293 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTATGTCTTA CATCTTTAGA TATTTTGTGA TAATTACAAT TAGTTTGGCT TACTTGAACA 60

AGATTCATTC CTCAAAATGA CCTGAACTGT TGAACATCAA AGGGGTTGAA ACATAGAGGA 120

AAACAACATG ATGAATGTTT CCATTGTCTA GGGATTTCTA TTATGTTGCT GAGAACAAAT 180

GTCATCTTAA AAAAAACATT GTTTACTTTT TTGTAGTATA GAAGATTACT GTATAGAGTT 240

TGCAAGTGTG TCTGTTTTGG AGTAATTGTG AAATGTTTGA TGAACTTGTA CAG 293

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 376 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GTTCAAGTAT TTTGAATCGC AGCTTGTTAA ATAATCTAGT AATTTTTAGA TTGCTTACTT 60

GGAAGTCTAC TTGGTTCTGG GGATGATAGC TCATTTCATC TTGTTCTACT TATTTTCCAA 120

CCGAATTTCT GATTTTTGTT TCGAGATCCA AGTATTAGAT TCATTTACAC TTATTACCGC 180

CTCATTTCTA CCACTAAGGC CTTGATGAGC AGCTTAAGTT GATTCTTTGA AGCTATAGTT 240

TCAGGCTACC AATCCACAGC CTGCTATATT TGTTGGATAC TTACCTTTTC TTTACAATGA 300

AGTGATACTA ATTGAAATGG TCTAAATCTG ATATCTATAT TTCTCCGTCT TTCCTCCCCC 360

TCATGATGAA ATGCAG 376

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTAAAATCAT CTAAAGTTGA AAGTGTTGGG TTTATGAAGT GCTTTAATTC TATCCAAGGA 60

CAAGTAGAAA CCTTTTTACC TTCCATTTCT TGATGATGGA TTTCATATTA TTTAATCCAA 120

TAGCTGGTCA AATTCGGTAA TAGCTGTACT GATTAGTTAC TTCACTTTGC AG 172

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 145 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GTATATATGT TTTACTTATC CATGAAATTA TTGCTCTGCT TGTTTTTAAT GTACTGAACA 60

AGTTTTATGG AGAAGTAACT GAAACAAATC ATTTTCACAT TGTCTAATTT AACTCTTTTT 120

TCTGATCCTC GCATGACGAA AACAG 145

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GTAAGGATTT GCTTGAATAA CTTTTGATAA TAAGATAACA GATGTAGGGT ACAGTTCTCT 60

CACCAAAAAG AACTGTAATT GTCTCATCCA TCTTTAGTTG TATAAGATAT CCGACTGTCT 120

GAGTTCGGAA GTGTTTGAGC CTCCTGCCCT CCCCCTGCGT TGTTTAGCTA ATTCAAAAAG 180

GAGAAAACTG TTTATTGATG ATCTTTGTCT TCATGCTGAC ATACAATCTG TTCTCATGAC 240

AG 242

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 797 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GTACAGTTCT TGCCGTGTGA CCTCCCTTTT TATTGTGGTT TTGTTCATAG TTATTTGAAT 60

GCGATAGAAG TTAACTATTG ATTACCGCCA CAATCGCCAG TTAAGTCCTC TGAACTACTA 120

ATTTGAAAGG TAGGAATAGC CGTAATAAGG TCTACTTTTG GCATCTTACT GTTACAAAAC 180

AAAAGGATGC CAAAAAAATT CTTCTCTATC CTCTTTTTCC CTAAACCAGT GCATGTAGCT 240

TGCACCTGCA TAAACTTAGG TAAATGATCA AAAATGAAGT TGATGGGAAC TTAAAACCGC 300

CCTGAAGTAA AGCTAGGAAT AGTCATATAA TGTCCACCTT TGGTGTCTGC GCTAACATCA 360

ACAACAACAT ACCTCGTGTA GTCCCACAAA GTGGTTTCAG GGGGAGGGTA GAGTGTATGC 420

AAAACTTACT CCTATCTCAG AGGTAGAGAG GATTTTTTCA ATAGACCCTT GGCTCAAGAA 480

AAAAAGTCCA AAAAGAAGTA ACAGAAGTGA AAGCAACATG TGTAGCTAAA GCGACCCAAC 540

TTGTTTGGGA CTGAAGTAGT TGTTGTTGTT GAAACAGTGC ATGTAGATGA ACACATGTCA 600

GAAAATGGAC AACACAGTTA TTTTGTGCAA GTCAAAAAAA TGTACTACTA TTTCTTTGTG 660

CAGCTTTATG TATAGAAAAG TTAAATAACT AATGAATTTT GCTAGCAGAA AAATAGCTTG 720

GAGAGAAATT TTTTATATTG AACTAAGCTA ACTATATTCA TCTTTCTTTT TGCTTCTTCT 780

TCTCCTTGTT TGTGAAG 797

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2169 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

ATCATGGCCA ATTACTGGTT CAAATGCATT ACTTCCTTTC AGATTCTTTC GAGTTCTCAT 60

GACCGGTCCT ACTACAGACG ATACTAACCC GTGGAACTGT TGCATCTGCT TCTTAGAACT 120

CTATGGCTAT TTTCGTTAGC TTGGCGTCGG TTTGAACATA GTTTTTGTTT TCAAACTCTT 180

CATTTACAGT CAAAATGTTG TATGGTTTTT GTTTTCCTCA ATGATGTTTA CAGTGTTGTG 240

TTGTCATCTG TACTTTTGCC TATTACTTGT TTTGAGTTAC ATGTTAAAAA AGTGTTTATT 300

TTGCCATATT TTGTTCTCTT ATTATTATTA TCATACATAC ATTATTACAA GGAAAAGACA 360

AGTACACAGA TCTTAACGTT TATGTTCAAT CAACTTTTGG AGGCATTGAC AGGTACCACA 420

AATTTTGAGT TTATGATTAA GTTCAATCTT AGAATATGAA TTTAACATCT ATTATAGATG 480

CATAAAAATA GCTAATGATA GAACATTGAC ATTTGGCAGA GCTTAGGGTA TGGTATATCC 540

AACGTTAATT TAGTAATTTT TGTTACGTAC GTATATGAAA TATTGAATTA ATCACATGAA 600

CGGTGGATAT TATATTATGA GTTGGCATCA GCAAAATCAT TGGTGTAGTT GACTGTAGTT 660

GCAGATTTAA TAATAAAATG GTAATTAACG GTCGATATTA AAATAACTCT CATTTCAAGT 720

GGGATTAGAA CTAGTTATTA AAAAAATGTA TACTTTAAGT GATTTGATGG CATATAATTT 780

AAAGTTTTTC ATTTCATGCT AAAATTGTTA ATTATTGTAA TGTAGACTGC GACTGGAATT 840

ATTATAGTGT AAATTTATGC ATTCAGTGTA AAATTAAAGT ATTGAACTTG TCTGTTTTAG 900

AAAATACTTT ATACTTTAAT ATAGGATTTT GTCATGCGAA TTTAAATTAA TCGATATTGA 960

ACACGGAATA CCAAAATTAA AAAGGATACA CATGGCCTTC ATATGAACCG TGAACCTTTG 1020

ATAACGTGGA AGTTCAAAGA AGGTAAAGTT TAAGAATAAA CTGACAAATT AATTTCTTTT 1080

ATTTGGCCCA CTACTAAATT TGCTTTACTT TCTAACATGT CAAGTTGTGC CCTCTTAGTT 1140

GAATGATATT CATTTTTCAT CCCATAAGTT CAATTTGATT GTCATACCAC CCATGATGTT 1200

CTGAAAAATG CTTGGCCATT CACAAAGTTT ATCTTAGTTC CTATGAACTT TATAAGAAGC 1260

TTTAATTTGA CATGTTATTT ATATTAGATG ATATAATCCA TGACCCAATA GACAAGTGTA 1320

TTAATATTGT AACTTTGTAA TTGAGTGTGT CTACATCTTA TTCAATCATT TAAGGTCATT 1380

AAAATAAATT ATTTTTTGAC ATTCTAAAAC TTTAAGCAGA ATAAATAGTT TATCAATTAT 1440

TAAAAACAAA AAACGACTTA TTTATAAATC AACAAACAAT TTTAGATTGC TCCAACATAT 1500

TTTTCCAAAT TAAATGCAGA AAATGCATAA TTTTATACTT GATCTTTATA GCTTATTTTT 1560

TTTAGCCTAA CCAACGAATA TTTGTAAACT CACAACTTGA TTAAAAGGGA TTTACAACAA 1620

GATATATATA AGTAGTGACA AATCTTGATT TTAAATATTT TAATTTGGAG GTCAAAATTT 1680

TACCATAATC ATTTGTATTT ATAATTAAAT TTTAAATATC TTATTTATAC ATATCTAGTA 1740

AACTTTTAAA TATACGTATA TACAAAATAT AAAATTATTG GCGTTCATAT TAGGTCAATA 1800

AATCCTTAAC TATATCTGCC TTACCACTAG GAGAAAGTAA AAAACTCTTT ACCAAAAATA 1860

CATGTATTAT GTATACAAAA AGTCGATTAG ATTACCTAAA TAGAAATTGT ATAACGAGTA 1920

AGTAAGTAGA AATATAAAAA AACTACAATA CTAAAAAAAA TATGTTTTAC TTCAATTTCG 1980

AAACTAATGG GGTCTGAGTG AAATATTCAG AAAGGGGAGG ACTAACAAAA GGGTCATAAT 2040

GTTTTTTTAT AAAAAGCCAC TAAAATGAGG AAATCAAGAA TCAGAACATA CAAGAAGGCA 2100

GCAGCTGAAG CAAAGTACCA TAATTTAATC AATGGAAATT AATTTCAAAG TTTTATCAAA 2160

ACCCATTCG 2169

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1165 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

CTGTCAAAGA AATTCTCGAG GTTACATGGA TATCTTGAGA ACTTAAGAAA TTTTACAGTA 60

TAATTGAACA AGTATATGCA GCATATCCTA ATTTCTGGAC TGACTGGTAG CCATAAACTG 120

AATTTGAATT CATAGAAATT ATTGGAGTAG CGTTTGAGCT TCTCAAGGTC CATACAAAGA 180

ACACATTCTC AACTATCCGT CTCATAGGAT ACAACATTTT CAATTGCAGT TCAACACCAA 240

AAAAATGTAA AAAATAGAAA CATCATGACC AGGTAATCAA AACATACTCG TTCGATACGG 300

AATCTATTAT TGGTACATTT AAAAGGCTAG AAAAAACAAA CTTCAGTAGC TATCTCAGCA 360

TTATAACTTA TTATGTTTCC AGCAAAAGCC ATAACAAATC TTATATAACT TTCACAAAGA 420

AACAATTTTT ATCATATCCC TGGACATATA ATGAACCCTT TATGTGTTCA GAACTTTGCC 480

CTTGACCATG TATTTGTGTT GTAAAAAATC CACTTATTAT GTATACATAA TTGATTTACA 540

ACAACAAACA CAATGTAATC CCACAAGTGG AGTGTGGTGA GGACTTTACC CCTACCTTAC 600

GAGATAGAGA GATTGTTTCT AATAGACCCT CGGCTAAAGT AAAAGCATTT CAAAGCAACG 660

CGAATATAAA GAAGGCATGA TAAAACACTA AAGGAAGCAT GCTAGAGCAT TCTTACCGAG 720

GAACAATAAC TACGACAAGA TATATAATAC AATAATCGAA GTACAAGAAA CAGAAAATAG 780

AATAACAAAG ATCAAATAAC AAAACAAGAA ACTACCCAAA TAATTCCACG ACTACTAGTA 840

TGAAAGGATA AGCCAGACAA CACTCAAATA CCTAACTAAC CTTCTACCCC TCATCCGTGT 900

CCTCCATAAC CTCCTAGAAC ACTCTTTCTA AATATTGTCT TCCCCCACCC CCCCTCCATC 960

TCTCAATTTT TGAATTTTAT ACACTCAACC ACCTTGCAAA TTTGTCACAT GATACTTACA 1020

TATGGCTCTA CAAGTGTCAT TTTTCTTCCA TATTTGATAT TATAAAAAAT AAAATAAAAA 1080

ACTAAGGAGA TGATCCAGAT ATATTGGAAA ATGAAATGCA AAGGCTAAAA ATAATTGAAA 1140

TTAACATGAA ATTAGTAAAA ATTAC 1165

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 317 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GCAAGCAATG CACCACAGTT AGTTTATATC AAAAAGAAGA AAGGTATTAA CGGAGCTAAA 60

AACTGTTATA TACCACATGA AAGAAGTTGA TAATGTGAAA ACACCATGCT CATAAAGATT 120

GTAATTCAAA TAACAAATGC CCACAGGAGT AAAGAGCTGT CTTTCCCAAG TTAAGGTATT 180

ATAAATTGGC GGAACGAAGT AACACATGTT TGACATCTCC ACACGGTGCA CAGATCAAAT 240

ATGCCATGAG CACCAGTCCA GAAGTTTTCC AACTATTTAT ATACTATCCA TGCAACCATA 300

TAAATTATCA AACATAC 317

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 504 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

CTGCAAAAAA AGAGAGCAGT TTACACAAGA AAAAACTGCT AAATCTCAAC AAAAGTATCA 60

TGAATTTAAT ATTAAGGAAG CTATTTCGAA CAGAAAGAGT AACTCATGAT AATAGAAGGA 120

AATTGTGAAG CAACAGAAGG AAGACTTTCT TTATTTCTAC AAAATTGCTT TAAGACTATA 180

TTTGATGCTT GTATAGTACA TGTTGAATCC CCTCAGCTTC TTTATGTCTA TACTTTTTTT 240

ATATTTTGAA TCTCCTTAGT GAAAATCTTT GCTTTGCCAC TGACACTCCG GGGGTGTGTC 300

ACTTCTCCAA AAACCTTGTC TACTTTTTTG AAGACCCAAT CAAACAGCTT TTTAAAAGAT 360

CAAAAAAATG GCCAGGTGCC ACCTAAATGG AGCCACTACT TACTCCCCGG TATGCAAAAT 420

TCTCTAGCAA AGTCAAAGTA GGTATAAACA ATTCATCTTC CAAAATAAGG TCAAACTGCC 480

TAAAGCACAA CTTTTGGCTG TTAC 504

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 146 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CTGCATTGTG GATGAGTTAA TTAGAAGCAT AACCTTAATA GCAATTAGAA CATGTAAGAA 60

AGCCAATGAT GCTGCAACAT CATGCTTTAA TAGGAAAATC TGTTATGATG ATGGAAACTA 120

CTATTTTGTA GTAGACGAGG ACCTAC 146

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 218 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CTGTTTAATT GCTGAAGTAG TAAGTTCTCA AGCACTTATA GAATTGACTC ATTTTGTTAA 60

GGGAAAGAGT ATGGGATCAA GTCCAAATTA GTAAAGACAC AATTATTTTA ACTTTTGCAT 120

TTCAAAATGT CTTACATAAC AAGACTAGTA AGAACATGAA TCGAAATGCC TGTGATGATG 180

GTGTTCAAAA TTCAGCTTCA AGGTATGAAT AACAAAAC 218

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

CTGTATCCAG CAGACATAAT AGGAGTGAAC ATAAAAATGT CACTGGATAA ATAACTTATC 60

ATGATATTCA GCGGCTACCA ATATTCTGAA GGCCCATGGC GAAAATAAGT ACTTTTATAC 120

TTTCAGGACG TATATATTTG GATTCTATCT AACAATTGTT CTGAGAATTA TTTAGTTGTA 180

GAAATAAATT TAAAATAC 198

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 208 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CTGTGGTTAG AAGCTAAAAG TGAATAGATG AGAAAAATTA CCTCCAAATA AGAGGGATAT 60

TGAAAAAGAA ACACAATGCA TGAAAAGAAT AAACAAATGA TAAACGAGAA AATTGAATAA 120

TCCATCAGAA CCCTGGTTAC CTCACAAAGA GTGAGATTTT CCGTGGCTAA CCTATATGAA 180

CCTTAAAATG CAATAGAAAC AGACAAAC 208

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 293 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CTGTACAAGT TCATCAAACA TTTCACAATT ACTCCAAAAC AGACACACTT GCAAACTCTA 60

TACAGTAATC TTCTATACTA CAAAAAAGTA AACAATGTTT TTTTTAAGAT GACATTTGTT 120

CTCAGCAACA TAATAGAAAT CCCTAGACAA TGGAAACATT CATCATGTTG TTTTCCTCTA 180

TGTTTCAACC CCTTTGATGT TCAACAGTTC AGGTCATTTT GAGGAATGAA TCTTGTTCAA 240

GTAAGCCAAA CTAATTGTAA TTATCACAAA ATATCTAAAG ATGTAAGACA TAC 293

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 376 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CTGCATTTCA TCATGAGGGG GAGGAAAGAC GGAGAAATAT AGATATCAGA TTTAGACCAT 60

TTCAATTAGT ATCACTTCAT TGTAAAGAAA AGGTAAGTAT CCAACAAATA TAGCAGGCTG 120

TGGATTGGTA GCCTGAAACT ATAGCTTCAA AGAATCAACT TAAGCTGCTC ATCAAGGCCT 180

TAGTGGTAGA AATGAGGCGG TAATAAGTGT AAATGAATCT AATACTTGGA TCTCGAAACA 240

AAAATCAGAA ATTCGGTTGG AAAATAAGTA GAACAAGATG AAATGAGCTA TCATCCCCAG 300

AACCAAGTAG ACTTCCAAGT AAGCAATCTA AAAATTACTA GATTATTTAA CAAGCTGCGA 360

TTCAAAATAC TTGAAC 376

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 172 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CTGCAAAGTG AAGTAACTAA TCAGTACAGC TATTACCGAA TTTGACCAGC TATTGGATTA 60

AATAATATGA AATCCATCAT CAAGAAATGG AAGGTAAAAA GGTTTCTACT TGTCCTTGGA 120

TAGAATTAAA GCACTTCATA AACCCAACAC TTTCAACTTT AGATGATTTT AC 172

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 145 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CTGTTTTCGT CATGCGAGGA TCAGAAAAAA GAGTTAAATT AGACAATGTG AAAATGATTT 60

GTTTCAGTTA CTTCTCCATA AAACTTGTTC AGTACATTAA AAACAAGCAG AGCAATAATT 120

TCATGGATAA GTAAAACATA TATAC 145

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 242 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CTGTCATGAG AACAGATTGT ATGTCAGCAT GAAGACAAAG ATCATCAATA AACAGTTTTC 60

TCCTTTTTGA ATTAGCTAAA CAACGCAGGG GGAGGGCAGG AGGCTCAAAC ACTTCCGAAC 120

TCAGACAGTC GGATATCTTA TACAACTAAA GATGGATGAG ACAATTACAG TTCTTTTTGG 180

TGAGAGAACT GTACCCTACA TCTGTTATCT TATTATCAAA AGTTATTCAA GCAAATCCTT 240

AC 242

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 797 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

CTTCACAAAC AAGGAGAAGA AGAAGCAAAA AGAAAGATGA ATATAGTTAG CTTAGTTCAA 60

TATAAAAAAT TTCTCTCCAA GCTATTTTTC TGCTAGCAAA ATTCATTAGT TATTTAACTT 120

TTCTATACAT AAAGCTGCAC AAAGAAATAG TAGTACATTT TTTTGACTTG CACAAAATAA 180

CTGTGTTGTC CATTTTCTGA CATGTGTTCA TCTACATGCA CTGTTTCAAC AACAACAACT 240

ACTTCAGTCC CAAACAAGTT GGGTCGCTTT AGCTACACAT GTTGCTTTCA CTTCTGTTAC 300

TTCTTTTTGG ACTTTTTTTC TTGAGCCAAG GGTCTATTGA AAAAATCCTC TCTACCTCTG 360

AGATAGGAGT AAGTTTTGCA TACACTCTAC CCTCCCCCTG AAACCACTTT GTGGGACTAC 420

ACGAGGTATG TTGTTGTTGA TGTTAGCGCA GACACCAAAG GTGGACATTA TATGACTATT 480

CCTAGCTTTA CTTCAGGGCG GTTTTAAGTT CCCATCAACT TCATTTTTGA TCATTTACCT 540

AAGTTTATGC AGGTGCAAGC TACATGCACT GGTTTAGGGA AAAAGAGGAT AGAGAAGAAT 600

TTTTTTGGCA TCCTTTTGTT TTGTAACAGT AAGATGCCAA AAGTAGACCT TATTACGGCT 660

ATTCCTACCT TTCAAATTAG TAGTTCAGAG GACTTAACTG GCGATTGTGG CGGTAATCAA 720

TAGTTAACTT CTATCGCATT CAAATAACTA TGAACAAAAC CACAATAAAA AGGGAGGTCA 780

CACGGCAAGA ACTGTAC 797

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2169 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CGAATGGGTT TTGATAAAAC TTTGAAATTA ATTTCCATTG ATTAAATTAT GGTACTTTGC 60

TTCAGCTGCT GCCTTCTTGT ATGTTCTGAT TCTTGATTTC CTCATTTTAG TGGCTTTTTA 120

TAAAAAAACA TTATGACCCT TTTGTTAGTC CTCCCCTTTC TGAATATTTC ACTCAGACCC 180

CATTAGTTTC GAAATTGAAG TAAAACATAT TTTTTTTAGT ATTGTAGTTT TTTTATATTT 240

CTACTTACTT ACTCGTTATA CAATTTCTAT TTAGGTAATC TAATCGACTT TTTGTATACA 300

TAATACATGT ATTTTTGGTA AAGAGTTTTT TACTTTCTCC TAGTGGTAAG GCAGATATAG 360

TTAAGGATTT ATTGACCTAA TATGAACGCC AATAATTTTA TATTTTGTAT ATACGTATAT 420

TTAAAAGTTT ACTAGATATG TATAAATAAG ATATTTAAAA TTTAATTATA AATACAAATG 480

ATTATGGTAA AATTTTGACC TCCAAATTAA AATATTTAAA ATCAAGATTT GTCACTACTT 540

ATATATATCT TGTTGTAAAT CCCTTTTAAT CAAGTTGTGA GTTTACAAAT ATTCGTTGGT 600

TAGGCTAAAA AAAATAAGCT ATAAAGATCA AGTATAAAAT TATGCATTTT CTGCATTTAA 660

TTTGGAAAAA TATGTTGGAG CAATCTAAAA TTGTTTGTTG ATTTATAAAT AAGTCGTTTT 720

TTGTTTTTAA TAATTGATAA ACTATTTATT CTGCTTAAAG TTTTAGAATG TCAAAAAATA 780

ATTTATTTTA ATGACCTTAA ATGATTGAAT AAGATGTAGA CACACTCAAT TACAAAGTTA 840

CAATATTAAT ACACTTGTCT ATTGGGTCAT GGATTATATC ATCTAATATA AATAACATGT 900

CAAATTAAAG CTTCTTATAA AGTTCATAGG AACTAAGATA AACTTTGTGA ATGGCCAAGC 960

ATTTTTCAGA ACATCATGGG TGGTATGACA ATCAAATTGA ACTTATGGGA TGAAAAATGA 1020

ATATCATTCA ACTAAGAGGG CACAACTTGA CATGTTAGAA AGTAAAGCAA ATTTAGTAGT 1080

GGGCCAAATA AAAGAAATTA ATTTGTCAGT TTATTCTTAA ACTTTACCTT CTTTGAACTT 1140

CCACGTTATC AAAGGTTCAC GGTTCATATG AAGGCCATGT GTATCCTTTT TAATTTTGGT 1200

ATTCCGTGTT CAATATCGAT TAATTTAAAT TCGCATGACA AAATCCTATA TTAAAGTATA 1260

AAGTATTTTC TAAAACAGAC AAGTTCAATA CTTTAATTTT ACACTGAATG CATAAATTTA 1320

CACTATAATA ATTCCAGTCG CAGTCTACAT TACAATAATT AACAATTTTA GCATGAAATG 1380

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TCTAATCCCA CTTGAAATGA GAGTTATTTT AATATCGACC GTTAATTACC ATTTTATTAT 1500

TAAATCTGCA ACTACAGTCA ACTACACCAA TGATTTTGCT GATGCCAACT CATAATATAA 1560

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CTCAAAATTT GTGGTACCTG TCAATGCCTC CAAAAGTTGA TTGAACATAA ACGTTAAGAT 1800

CTGTGTACTT GTCTTTTCCT TGTAATAATG TATGTATGAT AATAATAATA AGAGAACAAA 1860

ATATGGCAAA ATAAACACTT TTTTAACATG TAACTCAAAA CAAGTAATAG GCAAAAGTAC 1920

AGATGACAAC ACAACACTGT AAACATCATT GAGGAAAACA AAAACCATAC AACATTTTGA 1980

CTGTAAATGA AGAGTTTGAA AACAAAAACT ATGTTCAAAC CGACGCCAAG CTAACGAAAA 2040

TAGCCATAGA GTTCTAAGAA GCAGATGCAA CAGTTCCACG GGTTAGTATC GTCTGTAGTA 2100

GGACCGGTCA TGAGAACTCG AAAGAATCTG AAAGGAAGTA ATGCATTTGA ACCAGTAATT 2160

GGCCATGAT 2169

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11469 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

ATCATGGCCA ATTACTGGTT CAAATGCATT ACTTCCTTTC AGATTCTTTC GAGTTCTCAT 60

GACCGGTCCT ACTACAGACG ATACTAACCC GTGGAACTGT TGCATCTGCT TCTTAGAACT 120

CTATGGCTAT TTTCGTTAGC TTGGCGTCGG TTTGAACATA GTTTTTGTTT TCAAACTCTT 180

CATTTACAGT CAAAATGTTG TATGGTTTTT GTTTTCCTCA ATGATGTTTA CAGTGTTGTG 240

TTGTCATCTG TACTTTTGCC TATTACTTGT TTTGAGTTAC ATGTTAAAAA AGTGTTTATT 300

TTGCCATATT TTGTTCTCTT ATTATTATTA TCATACATAC ATTATTACAA GGAAAAGACA 360

AGTACACAGA TCTTAACGTT TATGTTCAAT CAACTTTTGG AGGCATTGAC AGGTACCACA 420

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AACGTTAATT TAGTAATTTT TGTTACGTAC GTATATGAAA TATTGAATTA ATCACATGAA 600

CGGTGGATAT TATATTATGA GTTGGCATCA GCAAAATCAT TGGTGTAGTT GACTGTAGTT 660

GCAGATTTAA TAATAAAATG GTAATTAACG GTCGATATTA AAATAACTCT CATTTCAAGT 720

GGGATTAGAA CTAGTTATTA AAAAAATGTA TACTTTAAGT GATTTGATGG CATATAATTT 780

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AAAATACTTT ATACTTTAAT ATAGGATTTT GTCATGCGAA TTTAAATTAA TCGATATTGA 960

ACACGGAATA CCAAAATTAA AAAGGATACA CATGGCCTTC ATATGAACCG TGAACCTTTG 1020

ATAACGTGGA AGTTCAAAGA AGGTAAAGTT TAAGAATAAA CTGACAAATT AATTTCTTTT 1080

ATTTGGCCCA CTACTAAATT TGCTTTACTT TCTAACATGT CAAGTTGTGC CCTCTTAGTT 1140

GAATGATATT CATTTTTCAT CCCATAAGTT CAATTTGATT GTCATACCAC CCATGATGTT 1200

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TACCATAATC ATTTGTATTT ATAATTAAAT TTTAAATATC TTATTTATAC ATATCTAGTA 1740

AACTTTTAAA TATACGTATA TACAAAATAT AAAATTATTG GCGTTCATAT TAGGTCAATA 1800

AATCCTTAAC TATATCTGCC TTACCACTAG GAGAAAGTAA AAAACTCTTT ACCAAAAATA 1860

CATGTATTAT GTATACAAAA AGTCGATTAG ATTACCTAAA TAGAAATTGT ATAACGAGTA 1920

AGTAAGTAGA AATATAAAAA AACTACAATA CTAAAAAAAA TATGTTTTAC TTCAATTTCG 1980

AAACTAATGG GGTCTGAGTG AAATATTCAG AAAGGGGAGG ACTAACAAAA GGGTCATAAT 2040

GTTTTTTTAT AAAAAGCCAC TAAAATGAGG AAATCAAGAA TCAGAACATA CAAGAAGGCA 2100

GCAGCTGAAG CAAAGTACCA TAATTTAATC AATGGAAATT AATTTCAAAG TTTTATCAAA 2160

ACCCATTCGA GGATCTTTTC CATCTTTCTC ACCTAAAGTT TCTTCAGGGG TAATTTTTAC 2220

TAATTTCATG TTAATTTCAA TTATTTTTAG CCTTTGCATT TCATTTTCCA ATATATCTGG 2280

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GGAGGTTATG GAGGACACGG ATGAGGGGTA GAAGGTTAGT TAGGTATTTG AGTGTTGTCT 2520

GGCTTATCCT TTCATACTAG TAGTCGTGGA ATTATTTGGG TAGTTTCTTG TTTTGTTATT 2580

TGATCTTTGT TATTCTATTT TCTGTTTCTT GTACTTCGAT TATTGTATTA TATATCTTGT 2640

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ATTGTGTTTG TTGTTGTAAA TCAATTATGT ATACATAATA AGTGGATTTT TTACAACACA 2880

AATACATGGT CAAGGGCAAA GTTCTGAACA CATAAAGGGT TCATTATATG TCCAGGGATA 2940

TGATAAAAAT TGTTTCTTTG TGAAAGTTAT ATAAGATTTG TTATGGCTTT TGCTGGAAAC 3000

ATAATAAGTT ATAATGCTGA GATAGCTACT GAAGTTTGTT TTTTCTAGCC TTTTAAATGT 3060

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TGATATAAAC TAACTGTGGT GCATTGCTTG CATGAAGCAC AGTTCAGCTA TTTCCGCTGT 3840

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AATGAAGAGA TATGTGGATC AGAAAATGCT CATTGAAAAA TATGAGGGAC CCCTTGAGGA 4020

ATTTGCTCAA GGTAACAGCC AAAAGTTGTG CTTTAGGCAG TTTGACCTTA TTTTGGAAGA 4080

TGAATTGTTT ATACCTACTT TGACTTTGCT AGAGAATTTT GCATACCGGG GAGTAAGTAG 4140

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GGAAGATGGT TGCATAGTCT ATCGTGAATG GGCTCCTGCT GCTCAGTAGG TCCTCGTCTA 4620

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CATCATTGGC TTTCTTACAT GTTCTAATTG CTATTAAGGT TATGCTTCTA ATTAACTCAT 4740

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GTAGATCGTA TCCCTGCTTG GATAAAGTAT GCCACTGCAG ACGCCACAAA GTTTGCAGCA 4980

CCATATGATG GTGTCTACTG GGACCCACCA CCTTCAGAAA GGTTTTGTTA TTCATACCTT 5040

GAAGCTGAAT TTTGAACACC ATCATCACAG GCATTTCGAT TCATGTTCTT ACTAGTCTTG 5100

TTATGTAAGA CATTTTGAAA TGCAAAAGTT AAAATAATTG TGTCTTTACT AATTTGGACT 5160

TGATCCCATA CTCTTTCCCT TAACAAAATG AGTCAATTCT ATAAGTGCTT GAGAACTTAC 5220

TACTTCAGCA ATTAAACAGG TACCACTTCA AATACCCTCG CCCTCCCAAA CCCCGAGCCC 5280

CACGAATCTA TGAAGCACAT GTCGGCATGA GCAGCTCTGA GCCACGTGTA AATTCGTATC 5340

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TCTAAAGTTG AAAGTGTTGG GTTTATGAAG TGCTTTAATT CTATCCAAGG ACAAGTAGAA 7680

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GAGAAGTAAC TGAAACAAAT CATTTTCACA TTGTCTAATT TAACTCTTTT TTCTGATCCT 7980

CGCATGACGA AAACAGGTAT AAAGTTGGAT GTGACTTGCC AGGGAAGTAC AGAGTTGCAC 8040

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CCCTTTTTAT TGTGGTTTTG TTCATAGTTA TTTGAATGCG ATAGAAGTTA ACTATTGATT 8520

ACCGCCACAA TCGCCAGTTA AGTCCTCTGA ACTACTAATT TGAAAGGTAG GAATAGCCGT 8580

AATAAGGTCT ACTTTTGGCA TCTTACTGTT ACAAAACAAA AGGATGCCAA AAAAATTCTT 8640

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TCCTCTTTTC TATTGATTTT CTTCATTGTT TTCTTCATTG TTGTGGTTGT TATTGAAAAG 11340

AAAGAAAATT TATAACAGAA AAAGATGTCA AAAAAAAGGT AAAATGAAAG AGTATCATAT 11400

ACTTAAAGAG TTGCGTAGAG ATAAGTCAAA AGAAACAGAA TTATAGTAAT TTCAGCTAAG 11460

TTAGAATTC 11469

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GGAATTCCAG TCGCAGTCTA CATTAC 26

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CGGGATCCAG AGGCATTAAG ATTTCTGG 28

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

CGGGATCCAA AGAAATTCTC GAGGTTACAT GG 32

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

CGGGATCCGG GGTAATTTTT ACTAATTTCA TG 32

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CGGGATCCCG TATGTCTCAC TGTGTTTGTG GC 32

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

CGGGATCCCC CTACATACAT ATATCAGATT AG 32

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

CCATCGATAC TTTAAGTGAT TTGATGGC 28

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Synthetic DNA Primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

CGGGATCCTG TTCTGATTCT TGATTTCC 28

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2122 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

GTATGTCTCA CTGTGTTTGT GGCTGTGTGT GTTTTTTTCT CTGTCTTTTT GTGTTTTGTG 60

TAATTGGGGC TCTTTAAAGT TGGTATTGTG TATACCCTTT TGAGTATAGT CTTTGAGGAA 120

GCAAAATGAT GAATCTTGAT TGACATTAGT AAGGGTTGTA ACTTTTTGAA GTTTGGTTAG 180

GTGTAATTGA GTTTGGCTTG TGTGTCTGTG TGTCGAGGTT ATTTTTTTGG TTTGTGTTAT 240

TGGGGATTCT TAAAAGTTGG TATTGTGTAT ACCCTTTTGA GTATAGTCTT TGAGGAAGCA 300

AAAATGATGA ATCTTGATTG GCATTAGTAA AGGTTGTAGC TTTTTGAAGT GTGGTTAGGT 360

GTAATTGAGT TTGGCTTGTG TGTCTGTGTG TTTTGGAATC CTGATGTGTG TCAAGTCCTG 420

ATATGGGTCG AGGTTCTTTC TTTGGTTTGT GTAATTGGGG GTTCTTAAAA GTTGGTATTA 480

TGTACCTTTT TAAGAATAGT GTCTGAGAAA GCAAAATCGA TGAATTTTGA TTGACAGCAT 540

ATTCTTTGAG AAAGCAAAAA ATGGTGAGTT TTCATGGAGA AACTTGATTG ACATTACTAA 600

AGGTAGCAAC TTTTTCAACT CCTGATATGG GTCAAGGTTC TTTGTTTGGT TTGTGTAATT 660

TGGGGTTCTT TGAAGTTTTG AGAAAGAAAA ATTATGATTT TTCATGGAGA AATTTGATTT 720

ACATTAATAA AGGTAGTAGC TTTTTAAAGT GTGGTCAGCT GTAATGAGTT CAGCTTGGTT 780

TAAAGGGGCC CTACATATGG TGCTTTCTGG TGAGATATTT GTTGCTCCAC CATACGAGTT 840

ATAAGAATCA TAGTGTTAGG ATCTTTTTTC TTTTTTTTTT CATTTTTCAC TTGACTAGCT 900

ACTAGAGGAG TGATCTTGAC GGCGGAAAAT CTTAGAAAGG GGAAGGTTGT TTGCATCAAC 960

TGGTGTTATA TGTGCAAGGA GACGGGAGAT GATGTAGATC ATCTTCTTCT TCATTGTGGT 1020

CTTTCCATGA GGTTATGATG TGATATGTTT GAATGGTTTG GTACTTCTTG GCTATGCCAA 1080

GAACTGTGAA AGAATTGATA TTCAGTTGGA AGTGTGGAGT TGGAAGAGTG GAAGAATTGA 1140

CACTTGGTTC CATTAGCTTT AATGTGGGTG GTGTGGAGAG AGAGAGAAAT AGGAGAGCTT 1200

TTGAGGGGGT AGAGTTGAGC TTTCCTCAGT TGAGAAGTAG CCTTTGATAT CTTTTTTTTT 1260

TTTTTTTGTA CACCCATAGA ATTCCCAATT GTATAGAAGA TTGGGTGGAG TTTGTAGAGA 1320

ATCATCTTTT GTAGTAGATT CTTTACCTTT TGGTATATCC ATTGTATACA GCCAGGCCTT 1380

TGACTATGTT TATGAATGAA TATACATTAC TTGAAAAAAA AAGAAGTGAA GCCAGTCTGT 1440

TGTACCTTTG TAGACAATGT TGTTGCAGCA TCTTGATAAT TCCCTGAAAA TTGTCTCCCT 1500

GAAGGAATAG TTTGGTTGAT ATTGATTATT TCTTGGTTTG TTTAATTCGG TGTTCTTGAA 1560

GGCCATTTTA AATCCTTTGA CATTGTTAAA GGTGTTTACA AGTGTTGGTC TGGGTTTAAA 1620

AGCACCTCTT GTATGGTGCT TTCTGGAGTG ATCTTTCTTC CTCCAAAAGA GAAGTTGCAA 1680

GAATCAGTGT GTGTACTTTT TTCTCTTGTA TGATCAGATC TTTTTTCAAT TTTTCCGTTT 1740

TAGTTGATTT ATCCATATAG TGAAAGTTGG TGTCATAGTT GCTGTTTGTG GACTTCCTGT 1800

AAAAGTTTTT TGATATACTT AAAAAATTGT CACACAGAAG AAAGAGTTTT TTACCATTAC 1860

TTAAGCTAGA TGGGACTGTT TGATTCTTAG ACCAAATAAT GAACCTTTTT GTTCTCTTAA 1920

CGTGTACTTG AAATAGTTTG GTAAAATTGT GATAGGAAAA AAGATAATTC TTGATTGCTT 1980

TTGGAGCATC ACTTCTAATC ATAAAAGTCT TTGCTCTCTT CAACCATGAA TGATAAATTG 2040

GACACTTATG TGGCCCTAAG TTGCTCTCAG TAGTGGTCTT TAATTGTGGA GATATAACTA 2100

ATCTGATATA TGTATGTAGG GA 2122

CLAIMS

1. A method of affecting enzymatic activity in a plant (or a cell, a tissue or an organ thereof) comprising expressing in the plant (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron of a class A potato starch branching enzyme in a sense orientation, optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in a sense or antisense orientation; and wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

2. A method according to claim 1 wherein starch branching enzyme activity is affected and/or wherein the levels of amylopectin are affected and/or the composition of starch is changed.

3. A method of affecting enzymatic activity in a starch producing organism (or a cell, a tissue or an organ thereof) comprising expressing in the starch producing organism (or a cell, a tissue or an organ thereof) a nucleotide sequence wherein the nucleotide sequence codes, partially or completely, for an intron of a class A starch branching enzyme in a sense orientation, optionally together with a nucleotide sequence which codes, partially or completely, for an intron of a class B starch branching enzyme in a sense or antisense orientation; wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron; and wherein starch branching enzyme activity is affected and/or the levels of amylopectin are affected and/or the composition of starch is changed.

4. A method according to any one of claims 1 to 3 wherein the nucleotide sequence does not contain a sequence that is sense to an exon sequence.

5. A method according to any one of the preceding claims wherein the enzymatic activity is reduced or eliminated.

6. A method according to any one of the preceding claims wherein the nucleotide sequence codes for at least substantially all of at least one intron in a sense orientation.

7. A method according to any one of the preceding claims wherein the nucleotide sequence codes for all of at least one intron in a sense orientation.

8. A method according to any one of the preceding claims wherein the nucleotide sequence comprises the sequence shown as SEQ. ID. No. 38, or a variant, derivative or homologue thereof.

9. A method according to any one of the preceding claims wherein the nucleotide sequence is expressed by a promoter having a sequence shown as SEQ.I.D. No. 14 or a variant, derivative or homologue thereof.

10. A promoter having a sequence shown as SEQ.I.D. No. 14, or a variant, derivative or homologue thereof.

11. A promoter according to claim 10 in combination with a gene of interest ("GOI").

12. A construct capable of comprising or expressing the invention according to any one of claims 10 and 11.

13. A vector comprising or expressing the invention according to any one of claims 10 to 12.

14. A combination of nucleotide sequences comprising a first nucleotide sequence coding for a recombinant enzyme; and a second nucleotide sequence which corresponds to a class A SBE intron in a sense orientation; wherein the intron is an intron that is associated with a genomic gene encoding an enzyme corresponding to the recombinant

enzyme; and wherein the second nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

15. A cell, tissue or organ comprising or expressing the invention according to any one of claims 10 to 14.

16. A transgenic starch producing organism comprising or expressing the invention according to any one of claims 10 to 15.

17. A transgenic starch producing organism according to claim 16 wherein the organism is a plant.

18. A starch obtained from the invention according to any one of the preceding claims.

19. A method of expressing a recombinant protein or enzyme in a host organism comprising expressing a nucleotide sequence coding for the recombinant protein or enzyme; and expressing a further nucleotide sequence; wherein the further nucleotide sequence codes, partially or completely, for a class A SBE intron in a sense orientation; wherein the intron is an intron normally associated with the genomic gene encoding a protein or an enzyme corresponding to the recombinant protein or enzyme; and wherein the further nucleotide sequence does not contain a sequence that is sense to an exon sequence normally associated with the intron.

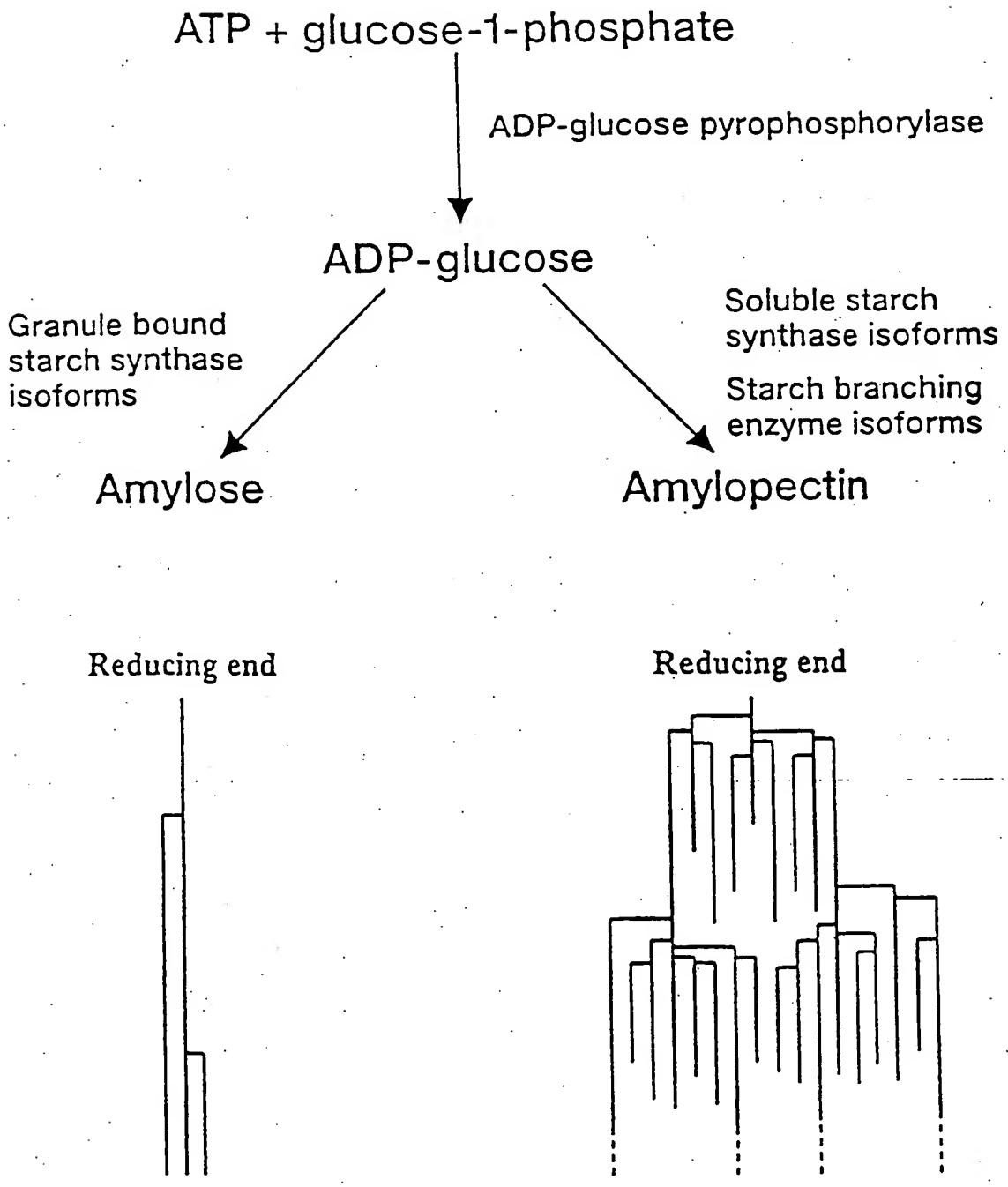
ATP + glucose-1-phosphate
ADP-glucose pyrophosphorylase
ADP-glucose
Granule bound starch synthase isoforms
Soluble starch synthase isoforms
Starch branching enzyme isoforms
Amylose
Amylopectin
Reducing end
Reducing end

FIG. 1

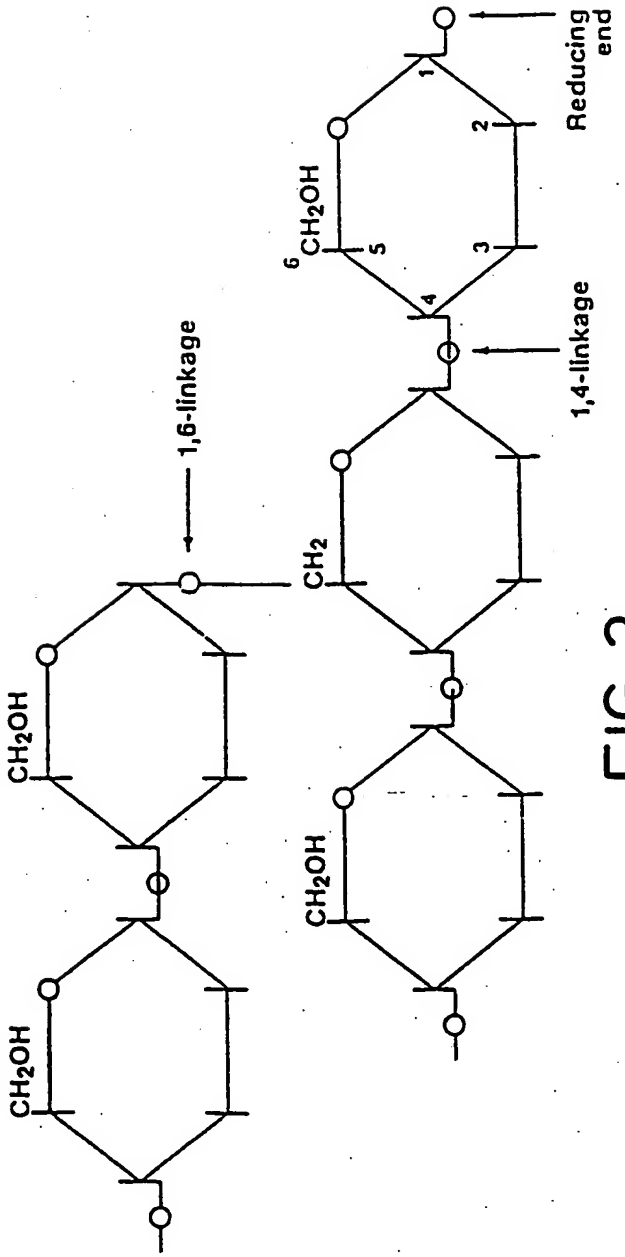
Reducing end
1,4-linkage
1,6-linkage
CH2OH
CH2
CH2OH
CH2OH
1
2
3
4
5
6

FIG. 2

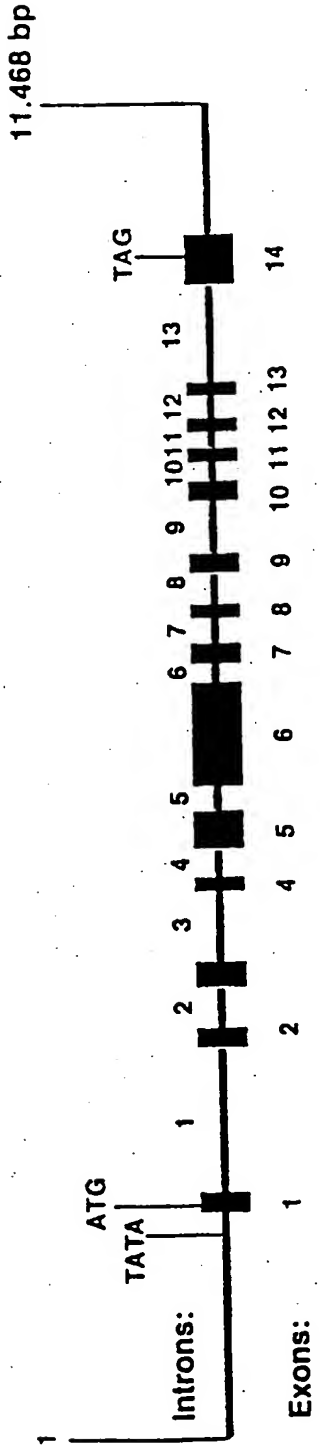

FIG. 3

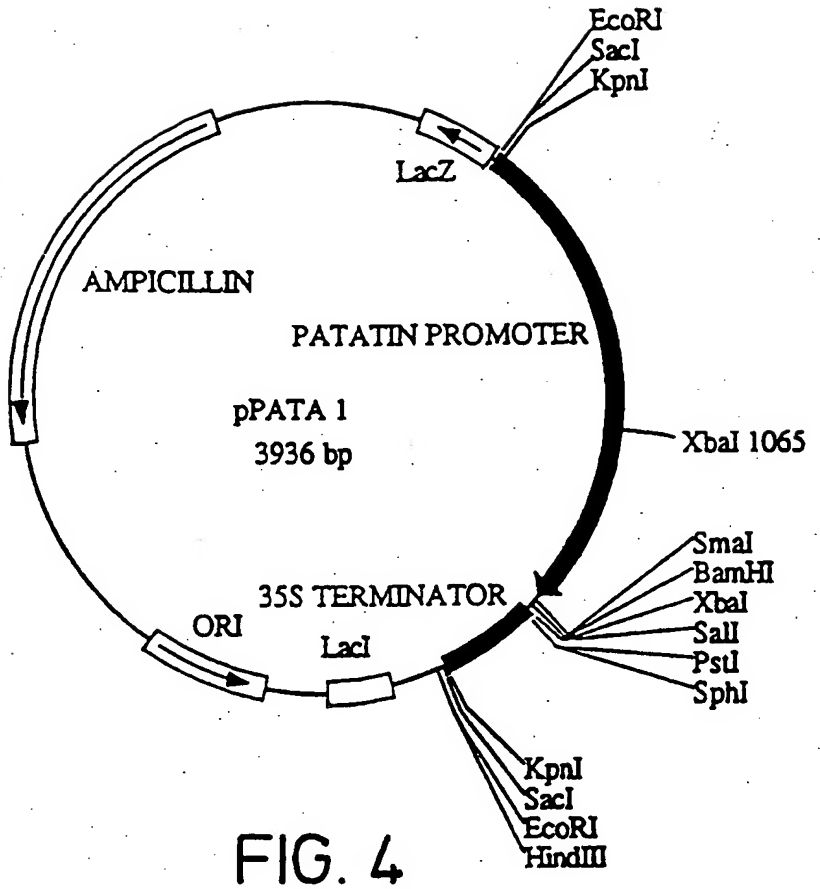
EcoRI
SacI
KpnI
LacZ
AMPICILLIN
PATATIN PROMOTER
pPATA 1
3936 bp
XbaI 1065
SmaI
BamHI
XbaI
SalI
PstI
SphI
35S TERMINATOR
ORI
LacI
KpnI
SacI
EcoRI
HindIII

FIG. 4

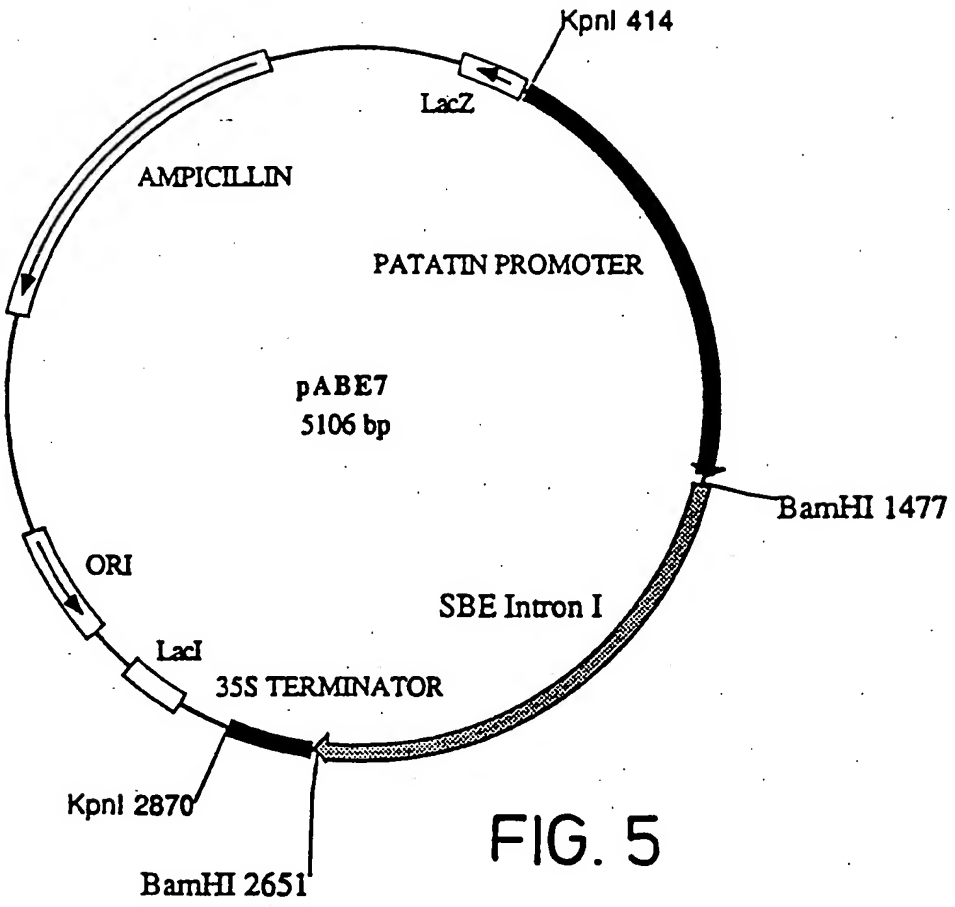
KpnI 414
LacZ
AMPICILLIN
PATATIN PROMOTER
pABE7
5106 bp
BamHI 1477
ORI
SBE Intron I
LacI
35S TERMINATOR
KpnI 2870
BamHI 2651

FIG. 5

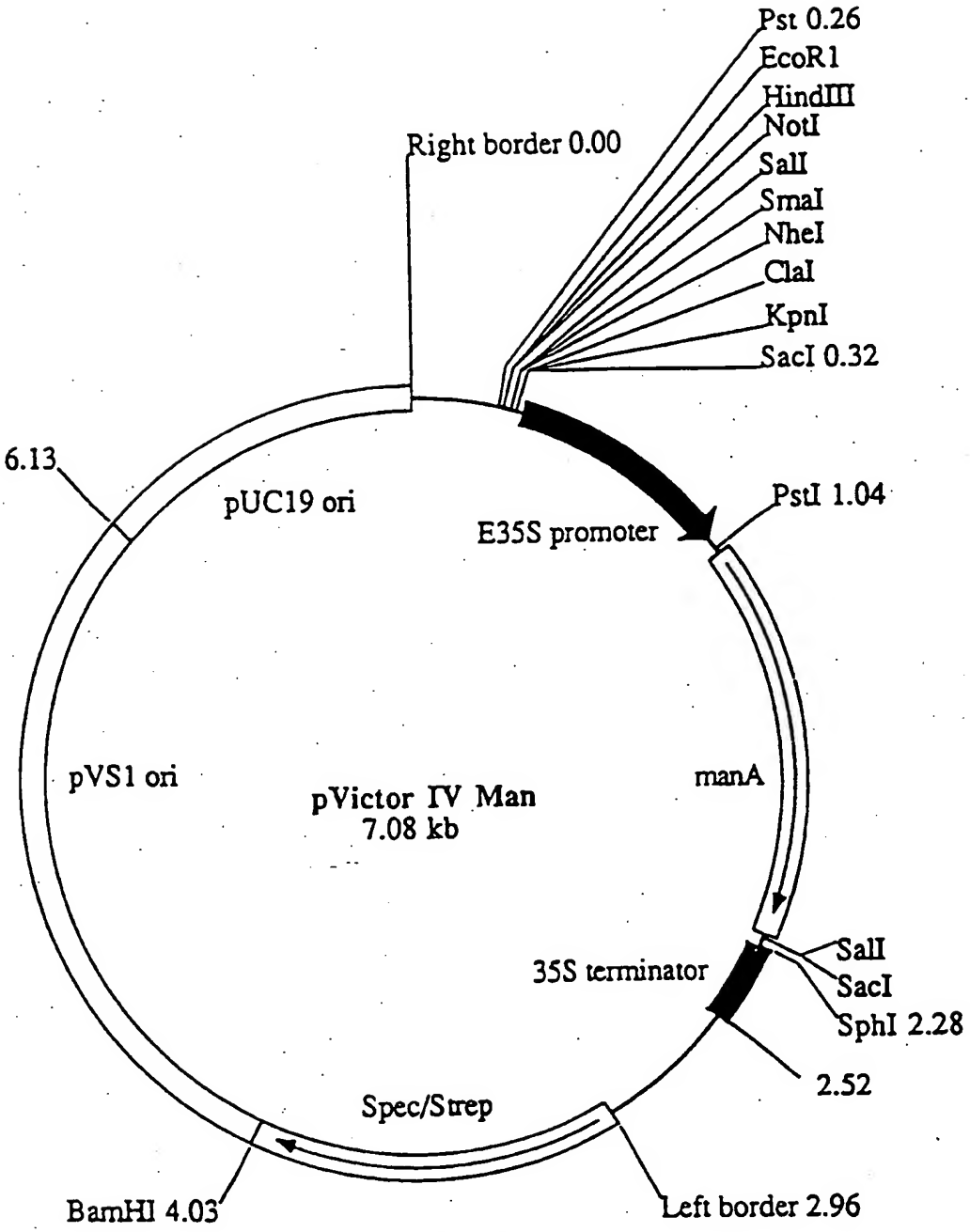
Pst 0.26
EcoR1
HindIII
NotI
SalI
SmaI
NheI
ClaI
KpnI
SacI 0.32
Right border 0.00
6.13
pUC19 ori
E35S promoter
PstI 1.04
pVS1 ori
pVictor IV Man
7.08 kb
manA
SalI
SacI
SphI 2.28
35S terminator
2.52
Spec/Strep
BamHI 4.03
Left border 2.96

FIG. 6

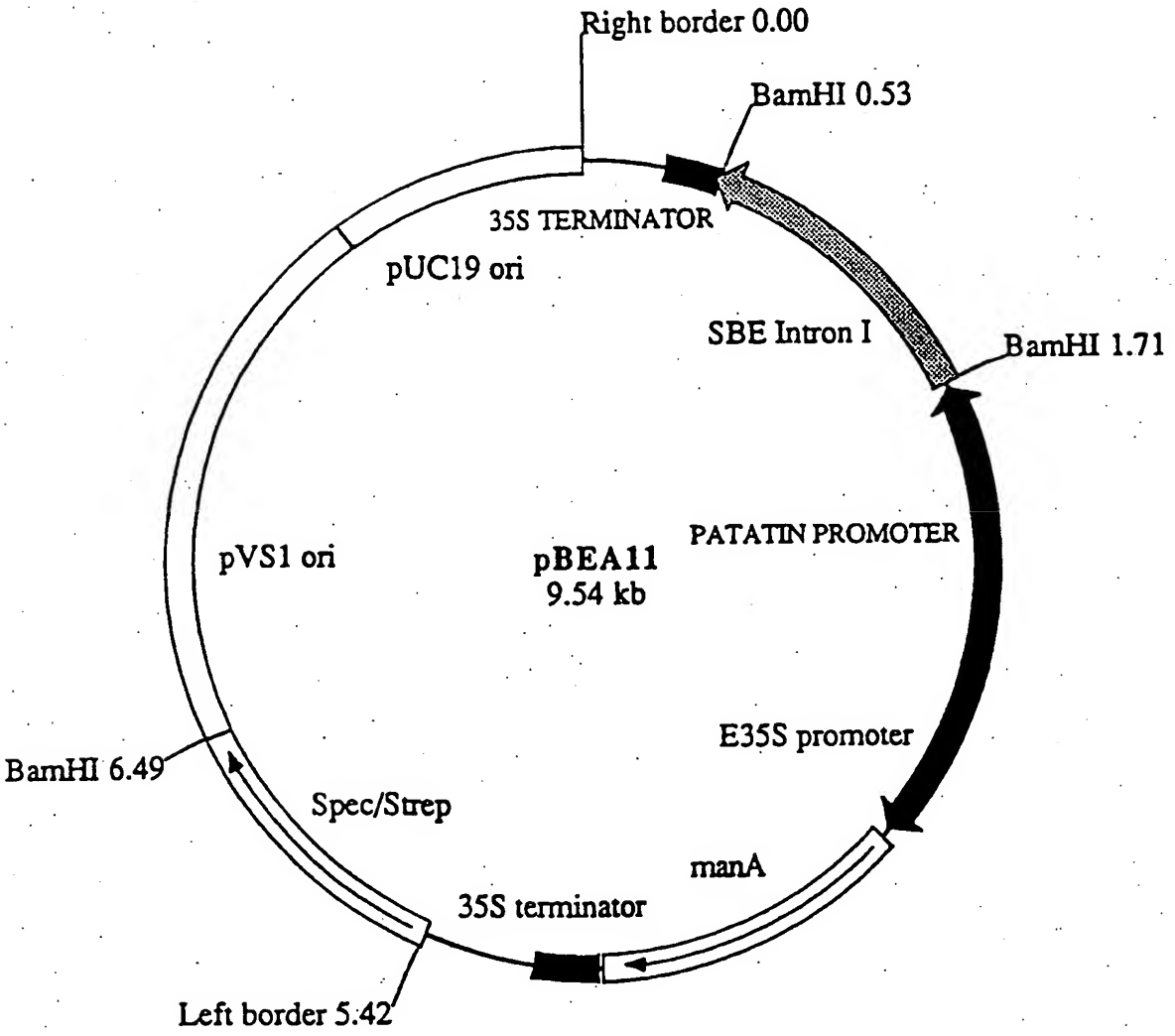
Right border 0.00
BamHI 0.53
35S TERMINATOR
pUC19 ori
SBE Intron I
BamHI 1.71
PATATIN PROMOTER
pVS1 ori
pBEA11
9.54 kb
E35S promoter
BamHI 6.49
Spec/Strep
manA
35S terminator
Left border 5.42

FIG. 7

```
      10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
ATCATGGCCAATTACTGGTTCAAATGCATTACTTCCTTTCAGATTCTTTCGAGTTCTCAT      60
GACCGGTCCTACTACAGACGATACTAACCCGTGGAACTGTTGCATCTGCTTCTTAGAACT     120
CTATGGCTATTTTCGTTAGCTTGGCGTCGGTTTGAACATAGTTTTTGTTTTCAAACTCTT     180
CATTTACAGTCAAAATGTTGTATGGTTTTTGTTTTCCTCAATGATGTTTACAGTGTTGTG     240
TTGTCATCTGTACTTTTGCCTATTACTTGTTTTGAGTTACATGTTAAAAAAGTGTTTATT     300
TTGCCATATTTTGTTCTCTTATTATTATTATCATACATACATTATTACAAGGAAAAGACA     360
AGTACACAGATCTTAACGTTTATGTTCAATCAACTTTTGGAGGCATTGACAGGTACCACA     420
AATTTTGAGTTTATGATTAAGTTCAATCTTAGAATATGAATTTAACATCTATTATAGATG     480
CATAAAAATAGCTAATGATAGAACATTGACATTTGGCAGAGCTTAGGGTATGGTATATCC     540
AACGTTAATTTAGTAATTTTTGTTACGTACGTATATGAAATATTGAATTAATCACATGAA     600
CGGTGGATATTATATTATGAGTTGGCATCAGCAAAATCATTGGTGTAGTTGACTGTAGTT     660
GCAGATTTAATAATAAAATGGTAATTAACGGTCGATATTAAAATAACTCTCATTTCAAGT     720
GGGATTAGAACTAGTTATTAAAAAAATGTATACTTTAAGTGATTTGATGGCATATAATTT     780
AAAGTTTTTCATTTCATGCTAAAATTGTTAATTATTGTAATGTAGACTGCGACTGGAATT     840
ATTATAGTGTAAATTTATGCATTCAGTGTAAAATTAAAGTATTGAACTTGTCTGTTTTAG     900
AAAATACTTTATACTTTAATATAGGATTTTGTCATGCGAATTTAAATTAATCGATATTGA     960
ACACGGAATACCAAAATTAAAAAGGATACACATGGCCTTCATATGAACCGTGAACCTTTG    1020
ATAACGTGGAAGTTCAAAGAAGGTAAAGTTTAAGAATAAACTGACAAATTAATTTCTTTT    1080
ATTTGGCCCACTACTAAATTTGCTTTACTTTCTAACATGTCAAGTTGTGCCCTCTTAGTT    1140
GAATGATATTCATTTTTCATCCCATAAGTTCAATTTGATTGTCATACCACCCATGATGTT    1200
CTGAAAAATGCTTGGCCATTCACAAAGTTTATCTTAGTTCCTATGAACTTTATAAGAAGC    1260
TTTAATTTGACATGTTATTTATATTAGATGATATAATCCATGACCCAATAGACAAGTGTA    1320
TTAATATTGTAACTTTGTAATTGAGTGTGTCTACATCTTATTCAATCATTTAAGGTCATT    1380
AAAATAAATTATTTTTTGACATTCTAAAACTTTAAGCAGAATAAATAGTTTATCAATTAT    1440
TAAAAACAAAAAACGACTTATTTATAAATCAACAAACAATTTTAGATTGCTCCAACATAT    1500
```

FIG. 8

```
         10        20        30        40        50        60
12345678901234567890123456789012345678901234567890123456789
TTTTCCAAATTAAATGCAGAAAATGCATAATTTTATACTTGATCTTTATAGCTTATTTTT  1560

TTTAGCCTAACCAACGAATATTTGTAAACTCACAACTTGATTAAAAGGGATTTACAACAA  1620

GATATATATAAGTAGTGACAAATCTTGATTTTAAATATTTTAATTTGGAGGTCAAAATTT  1680

TACCATAATCATTTGTATTTATAATTAAATTTTAAATATCTTATTTATACATATCTAGTA  1740

AACTTTTAAATATACGTATATACAAAATATAAAATTATTGGCGTTCATATTAGGTCAATA  1800

AATCCTTAACTATATCTGCCTTACCACTAGGAGAAAGTAAAAAACTCTTTACCAAAAATA  1860

CATGTATTATGTATACAAAAAGTCGATTAGATTACCTAAATAGAAATTGTATAACGAGTA  1920

AGTAAGTAGAAATATAAAAAAACTACAATACTAAAAAAAATATGTTTTACTTCAATTTCG  1980

AAACTAATGGGGTCTGAGTGAAATATTCAGAAAGGGGAGGACTAACAAAAGGGTCATAAT  2040

GTTTTTTTATGAAAAGCCACTAAAATGAGGAAATCAAGAATCAGAACATACAAGAAGGCA  2100

GCAGCTGAAGCAAAGTACCATAATTTAATCAATGGAAATTAATTTCAAAGTTTTATCAAA  2160
                                  M  E  I  N  F  K  V  L  S  K
ACCCATTCGAGGATCTTTTCCATCTTTCTCACCTAAAGTTTCTTCAGGGgtaatttttac  2220
 P  I  R  G  S  F  P  S  F  S  P  K  V  S  S  G
taatttcatgttaatttcaattatttttagcctttgcatttcattttccaatatatctgg  2280

atcatctccttagtttttttatttttatttttttataatatcaaatatggaagaaaaatgaca  2340

cttgtagagccatatgtaagtatcatgtgacaaatttgcaaggtggttgagtgtataaaa  2400

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caaatacatggtcaagggcaaagttctgaacacataaagggttcattatatgtccaggga  2940

tatgataaaaattgtttctttgtgaaagttatataagatttgttatggcttttgctggaa  3000
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FIG. 8 CONTINUED

```
         10        20        30        40        50        60
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acataataagttataatgctgagatagctactgaagtttgtttttctagccttttaaat  3060

gtaccaataatagattccgtatcgaacgagtatgttttgattacctggtcatgatgtttc  3120

tatttttacatttttttggtgttgaactgcaattgaaaatgttgtatcctatgagacgg  3180

atagttgagaatgtgttctttgtatggaccttgagaagctcaaacgctactccaataatt  3240

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tatacttgttcaattatactgtaaaatttcttaagttctcaagatatccatgtaacctcg  3360

agaatttctttgacagGCTTCTAGAAATAAGATATGTTTTCCTTCTCAACATAGTACTGG  3420
                A  S  R  N  K  I  C  F  P  S  Q  H  S  T  G
ACTGAAGTTTGGATCTCAGGAACGGTCTTGGGATATTTCTTCCACCCCAAAATCAAGAGT  3480
 L  K  F  G  S  Q  E  R  S  W  D  I  S  S  T  P  K  S  R  V
TAGAAAAGATGAAAGGgtatgtttgataatttatatggttgcatggatagtatataaata  3540
 R  K  D  E  R
gttggaaaacttctggactggtgctcatggcatatttgatctgtgcaccgtgtggagatg  3600

tcaaacatgtgttacttcgttccgccaatttataataccttaacttgggaaagacagctc  3660

tttactcctgtgggcatttgttatttgaattacaatctttatgagcatggtgttttcaca  3720

ttatcaacttctttcatgtggtatataacagttttagctccgttaataccttttcttctt  3780

tttgatataaactaactgtggtgcattgcttgcbkkkATGAAGCACAGTTCAGCTATTTC  3840
                                    M  K  H  S  S  A  I  S
CGCTGTTTTGACCGATGACGACAATTCGACAATGGCACCCCTAGAGGAAGATGTCAAGAC  3900
 A  V  L  T  D  D  D  N  S  T  M  A  P  L  E  E  D  V  K  T
TGAAAATATTGGCCTCCTAAATTTGGATCCAACTTTGGAACCTTATCTAGATCACTTCAG  3960
 E  N  I  G  L  L  N  L  D  P  T  L  E  P  Y  L  D  H  F  R
ACACAGAATGAAGAGATATGTGGATCAGAAAATGCTCATTGAAAAATATGAGGGACCCCT  4020
 H  R  M  K  R  Y  V  D  Q  K  M  L  I  E  K  Y  E  G  P  L
TGAGGAATTTGCTCAAGgtaacagccaaaagttgtgctttaggcagtttgaccttatttt  4080
 E  E  F  A  Q  G
ggaagatgaattgtttatacctactttgactttgctagagaattttgcataccggggagt  4140

aagtagtggctccatttaggtggcacctggccatttttttgatcttttaaaaagctgttt  4200

gattgggtcttcaaaaaagtagacaaggtttttggagaagtgacacacccccggagtgtc  4260

agtggcaaagcaaagattttcactaaggagattcaaaatataaaaaaagtatagacataa  4320

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FIG. 8 CONTINUED

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
tagcagttttttcttgtgtaaactgctctctttttttgcagGTTATTTAAAATTTGGATT          4560
                                                Y  L  K  F  G  F
CAACAGGGAAGATGGTTGCATAGTCTATCGTGAATGGGCTCCTGCTGCTCAgtaggtcct          4620
 N  R  E  D  G  C  I  V  Y  R  E  W  A  P  A  A  Q
cgtctactacaaaatagtagtttccatcatcataacagattttcctattaaagcatgatg          4680

ttgcagcatcattggctttcttacatgttctaattgctattaaggttatgcttctaatta          4740

actcatccacaatgcagGGAAGCAGAAGTTATTGGCGATTTCAATGGATGGAACGGTTCT          4800
                     E  A  E  V  I  G  D  F  N  G  W  N  G  S
AACCACATGATGGAGAAGGACCAGTTTGGTGTTTGGAGTATTAGAATTCCTGATGTTGAC          4860
N  H  M  M  E  K  D  Q  F  G  V  W  S  I  R  I  P  D  V  D
AGTAAGCCAGTCATTCCACACAACTCCAGAGTTAAGTTTCGTTTCAAACATGGTAATGGA          4920
S  K  P  V  I  P  H  N  S  R  V  K  F  R  F  K  H  G  N  G
GTGTGGGTAGATCGTATCCCTGCTTGGATAAAGTATGCCACTGCAGACGCCACAAAGTTT          4980
V  W  V  D  R  I  P  A  W  I  K  Y  A  T  A  D  A  T  K  F
GCAGCACCATATGATGGTGTCTACTGGGACCCACCACCTTCAGAAAGgttttgttattca          5040
A  A  P  Y  D  G  V  Y  W  D  P  P  P  S  E  R
taccttgaagctgaattttgaacaccatcatcacaggcatttcgattcatgttcttacta          5100

gtcttgttatgtaagacattttgaaatgcaaaagttaaaataattgtgtctttactaatt          5160

tggacttgatcccatactctttcccttaacaaaatgagtcaattctataagtgcttgaga          5220

acttactacttcagcaattaaacagGTACCACTTCAAATACCCTCGCCCTCCCAAACCCC          5280
                              Y  H  F  K  Y  P  R  P  P  K  P  R
GAGCCCCACGAATCTATGAAGCACATGTCGGCATGAGCAGCTCTGAGCCACGTGTAAATT          5340
  A  P  R  I  Y  E  A  H  V  G  M  S  S  S  E  P  R  V  N  S
CGTATCGTGAGTTTGCAGATGATGTTTTACCTCGGATTAAGGCAAATAACTATAATACTG          5400
  Y  R  E  F  A  D  D  V  L  P  R  I  K  A  N  N  Y  N  T  V
TCCAGTTGATGGCCATAATGGAACATTCTTACTATGGATCATTTGGATATCATGTTACAA          5460
  Q  L  M  A  I  M  E  H  S  Y  Y  G  S  F  G  Y  H  V  T  N
ACTTTTTTGCTGTGAGCAGTAGATATGGAAACCCGGAGGACCTAAAGTATCTGATAGATA          5520
  F  F  A  V  S  S  R  Y  G  N  P  E  D  L  K  Y  L  I  D  K
AAGCACATAGCTTGGGTTTACAGGTTCTGGTGGATGTAGTTCACAGTCATGCAAGCAATA          5580
  A  H  S  L  G  L  Q  V  L  V  D  V  V  H  S  H  A  S  N  N
ATGTCACTGATGGCCTCAATGGCTTTGATATTGGCCAAGGTTCTCAAGAATCCTACTTTC          5640
  V  T  D  G  L  N  G  F  D  I  G  Q  G  S  Q  E  S  Y  F  H
ATGCTGGAGAGCGAGGGTACCATAAGTTGTGGGATAGCAGGCTGTTCAACTATGCCAATT          5700
  A  G  E  R  G  Y  H  K  L  W  D  S  R  L  F  N  Y  A  N  W
GGGAGGTTCTTCGTTTCCTTCTTTCCAACTTGAGGTGGTGGCTAGAAGAGTATAACTTTG          5760
  E  V  L  R  F  L  L  S  N  L  R  W  W  L  E  E  Y  N  F  D
ACGGATTTCGATTTGATGGAATAACTTCTATGCTGTATGTTCATCATGGAATCAATATGG          5820
  G  F  R  F  D  G  I  T  S  M  L  Y  V  H  H  G  I  N  M  G
GATTTACAGGAAACTATAATGAGTATTTCAGCGAGGCTACAGATGTTGATGCTGTGGTCT          5880
  F  T  G  N  Y  N  E  Y  F  S  E  A  T  D  V  D  A  V  V  Y
ATTTAATGTTGGCCAATAATCTGATTCACAAGATTTTCCCAGATGCAACTGTTATTGCCG          5940
  L  M  L  A  N  N  L  I  H  K  I  F  P  D  A  T  V  I  A  E
AAGATGTTTCTGGTATGCCGGGCCTTGGCCGGCCTGTTTCTGAGGGAGGAATTGGTTTTG          6000
  D  V  S  G  M  P  G  L  G  R  P  V  S  E  G  G  I  G  F  V
```

FIG. 8 CONTINUED

```
         10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
TTTACCGCCTGGCAATGGCAATCCCAGATAAGTGGATAGATTATTTAAAGAATAAGAATG                6060
  Y  R  L  A  M  A  I  P  D  K  W  I  D  Y  L  K  N  K  N  D
ATGAAGATTGGTCCATGAAGGAAGTAACATCGAGTTTGACAAATAGGAGATATACAGAGA               6120
  E  D  W  S  M  K  E  V  T  S  S  L  T  N  R  R  Y  T  E  K
AGTGTATAGCATATGCGGAGACCCATGATCAGgtattttaaatttatttctacaactaaa               6180
  C  I  A  Y  A  E  T  H  D  Q
taattctcagaacaattgttagatagaatccaaatatatacgtcctgaaagtataaaagt               6240

acttatttttcgccatgggccttcagaatattggtagccgctgaatatcatgataagttat              6300

ttatccagtgacatttttatgttcactcctattatgtctgctggatacagTCTATTGTTG              6360
                                                    S  I  V  G
GTGACAAGACCATTGCATTTCTCCTAATGGACAAAGAGATGTATTCTGGCATGTCTTGCT              6420
  D  K  T  I  A  F  L  L  M  D  K  E  M  Y  S  G  M  S  C  L
TGACAGATGCTTCTCCTGTTGTTGATCGAGGAATTGCGCTTCACAAGgtttgtctgtttc              6480
  T  D  A  S  P  V  V  D  R  G  I  A  L  H  K
tattgcattttaaggttcatataggttagccacggaaaatctcactctttgtgaggtaac               6540

cagggttctgatggattattcaattttctcgtttatcatttgtttattcttttcatgcat               6600

tgtgtttctttttcaatatccctcttatttggaggtaatttttctcatctattcacttttt              6660

agcttctaaccacagATGATCCATTTTTTCACAATGGCCTTGGGAGGAGAGGGGTACCTC              6720
                  M  I  H  F  F  T  M  A  L  G  G  E  G  Y  L
AATTTCATGGGTAACGAGgtatgtcttacatctttagatattttgtgataattacaatta              6780
N  F  M  G  N  E
gtttggcttacttgaacaagattcattcctcaaaatgacctgaactgttgaacatcaaag               6840

gggttgaaacatagaggaaaacaacatgatgaatgtttccattgtctagggatttctatt               6900

atgttgctgagaacaaatgtcatcttaaaaaaaacattgtttacttttttgtagtatagta              6960

agattactgtatagagtttgcaagtgtgtctgttttggagtaattgtgaaatgtttgatg               7020

aacttgtacagTTTGGCCATCCTGAGTGGATTGACTTCCCTAGAGAGGGCAATAATTGGA              7080
            F  G  H  P  E  W  I  D  F  P  R  E  G  N  N  W  S
GTTATGACAAATGTAGACGCCAGTGGAACCTCGCGGATAGCGAACACTTGAGATACAAGg              7140
  Y  D  K  C  R  R  Q  W  N  L  A  D  S  E  H  L  R  Y  K
ttcaagtattttgaatcgcagcttgttaaataatctagtaatttttagattgcttacttg               7200

gaagtctacttggttctggggatgatagctcatttcatcttgttctacttatttttccaac              7260

cgaatttctgatttttgtttcgagatccaagtattagattcatttacacttattaccgcc               7320

tcatttctaccactaaggccttgatgagcagcttaagttgattctttgaagctatagttt               7380

caggctaccaatccacagcctgctatatttgttggatacttacctttttctttacaatgaa              7440

gtgatactaattgaaatggtctaaatctgatatctatatttctccgtctttcctcccccct              7500
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FIG. 8 CONTINUED

```
          10        20        30        40        50        60
1234567890123456789012345678901234567890123456789012345678901234567890
catgatgaaatgcagTTTATGAATGCATTTGATAGAGCTATGAATTCGCTCGATGAAAAG          7560
                  F  M  N  A  F  D  R  A  M  N  S  L  D  E  K
TTCTCATTCCTCGCATCAGGAAAACAGATAGTAAGCAGCATGGATGATGATAATAAGgta          7620
F  S  F  L  A  S  G  K  Q  I  V  S  S  M  D  D  D  N  K
aaatcatctaaagttgaaagtgttgggtttatgaagtgctttaattctatccaaggacaa          7680

gtagaaacctttttaccttccatttcttgatgatggatttcatattatttaatccaatag          7740

ctggtcaaattcggtaatagctgtactgattagttacttcactttgcagGTTGTTGTGTT          7800
                                                  V  V  V  F
TGAACGTGGTGACCTGGTATTTGTATTCAACTTCCACCCAAAGAACACATACGAAGGgta          7860
 E  R  G  D  L  V  F  V  F  N  F  H  P  K  N  T  Y  E  G
tatatgttttacttatccatgaaattattgctctgcttgtttttaatgtactgaacaagt          7920

tttatggagaagtaactgaaacaaatcattttcacattgtctaatttaactctttttttct          7980

gatcctcgcatgacgaaaacagGTATAAAGTTGGATGTGACTTGCCAGGGAAGTACAGAG          8040
                              Y  K  V  G  C  D  L  P  G  K  Y  R  V
TTGCACTGGACAGTGATGCTTGGGAATTTGGTGGCCATGGAAGAgtaaggatttgcttga          8100
  A  L  D  S  D  A  W  E  F  G  G  H  G  R
ataacttttgataataagataacagatgtagggtacagttctctcaccaaaaagaactgt          8160

aattgtctcatccatctttagttgtataagatatccgactgtctgagttcggaagtgttt          8220

gagcctcctgccctccccctgcgttgtttagctaattcaaaaaggagaaaactgtttatt          8280

gatgatctttgtcttcatgctgacatacaatctgttctcatgacagACTGGTCATGATGT          8340
                                                  T  G  H  D  V
TGACCATTTCACATCACCAGAAGGAATACCTGGAGTTCCAGAAACAAATTTCAATGGTCG          8400
 D  H  F  T  S  P  E  G  I  P  G  V  P  E  T  N  F  N  G  R
TCCAAATTCCTTCAAAGTGCTGTCTCCTGCGCGAACATGTGTGgtacagttcttgccgtg          8460
 P  N  S  F  K  V  L  S  P  A  R  T  C  V
tgacctccctttttattgtggttttgttcatagttatttgaatgcgatagaagttaacta          8520

ttgattaccgccacaatcgccagttaagtcctctgaactactaatttgaaaggtaggaat          8580

agccgtaataaggtctacttttggcatcttactgttacaaaacaaaaggatgccaaaaaa          8640

attcttctctatcctctttttccctaaaccagtgcatgtagcttgcacctgcataaactt          8700

aggtaaatgatcaaaaatgaagttgatgggaacttaaaaccgccctgaagtaaagctagg          8760

aatagtcatataatgtccacctttggtgtctgcgctaacatcaacaacaacatacctcgt          8820

gtagtcccacaaagtggtttcagggggagggtagagtgtatgcaaaacttactcctatct          8880

cagaggtagagaggatttttttcaatagacccttggctcaagaaaaaaagtccaaaaagaa          8940

gtaacagaagtgaaagcaacatgtgtagctaaagcgacccaacttgtttgggactgaagt          9000
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FIG. 8 CONTINUED

```
         10        20        30        40        50        60
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agttgttgttgttgaaacagtgcatgtagatgaacacatgtcagaaaatggacaacacag          9060

ttattttgtgcaagtcaaaaaaatgtactactatttctttgtgcagctttatgtatagaa          9120

aagttaaataactaatgaattttgctagcagaaaaatagcttggagagaaatttttttata         9180

ttgaactaagctaactatattcatctttctttttgcttcttcttctccttgtttgtgaag          9240

GCTTATTACAGAGTTGATGAACGCATGTCAGAAACTGAAGATTACCAGACAGACATTTGT          9300
A  Y  Y  R  V  D  E  R  M  S  E  T  E  D  Y  Q  T  D  I  C
AGTGAGCTACTACCAACAGCCAATATCGAGGAGAGTGACGAGAAACTTAAAGATTCGTTA          9360
S  E  L  L  P  T  A  N  I  E  E  S  D  E  K  L  K  D  S  L
TCTAGAAATATCAGTAACATTGACGAACGCATGTCAGAAACTGAAGTTTACCAGACAGAC          9420
S  T  N  I  S  N  I  D  E  R  M  S  E  T  E  V  Y  Q  T  D
ATTTCTAGTGAGCTACTACCAACAGCCAATATTGAGGAGAGTGACGAGAAACTTAAAGAT          9480
I  S  S  E  L  L  P  T  A  N  I  E  E  S  D  E  K  L  K  D
TCGTTATCTACAAATATCAGTAACATTGATCAGACTGTTGTAGTTTCTGTTGAGGAGAGA          9540
S  L  S  T  N  I  S  N  I  D  Q  T  V  V  V  S  V  E  E  R
GACAAGGAACTTAAAGATTCACCGTCTGTAAGCATCATTAGTGATGTTGTTCCAGCTGAA          9600
D  K  E  L  K  D  S  P  S  V  S  I  I  S  D  V  V  P  A  E
TGGGATGATTCAGATGCAAACGTCTGGGGTGAGGACTAGTCAGATGATTGATCGACCCTT          9660
W  D  D  S  D  A  N  V  W  G  E  D
CTACCGATTGGTGATCGCTATCCTTGCTCTCTGAGAAATAGGTGAGGCGAAACAAAAAAT          9720

AATTTGCATGATAAAAAGTCTGATTTTATGATCGCTATCCTCGCTCTCTGAGAAAGAAGC          9780

GAAACAAAGGCGACTCCTGGACTCGAATCTATAAGATAACAAAGGCGACTCCTGGGACTC          9840

GAATCTATAAGATAACAAAGGCAATTCCAAGACTTGAATCTATAAAAAATTTAGTTAAGA          9900

ATGATTAACGTCCGATCCTAATTCGAATCGAGGCATCTTACCACTCCATTGATAATTATA          9960

TAAGTCAATAAGTCATATAAWAGTATTAAAAACTAAATTGACTTGATCGGTCTATCAAAA         10020

ATMAGATMAAATTGTGTTCATATGTAACATTTTTGTTGTCACAATTAGCTTAATTACATC         10080

TTTCATGTGCAATAACAAAGAAATGATAGGAATTTAGAGATTCCAATTTTTTTGTTGCCA         10140

CAATTAACTTAATTACATCTTTCATTTGCAATAACAAAGAAATGATAGGAATTTAGAGAT         10200

CCAGTGTCAATACACAACCTAGGCCAACATCGAAAGCATAACTGTAAACTCATGCATGAA         10260

GAAATCAGTCGTAAAAATGAATAAATGCGACATAAAAACAAATTGCATGTATCATTAATG         10320

TGACTTAACTACAAGTAAAAATAAATTTAACAAATGTAACTTAACTACAAGTAAAAATAA         10380

ATTGCTTCTATCATTAACAAACAAACAGAATTAAAAAGAAAAAAACATACTAAATCTTAC         10440

CGTCATTCGATAAAAAAAAATACCAAATTCATAATGCAAGGAAAACGAAACGCGTCCTGA         10500
```

FIG. 8 CONTINUED

```
         10        20        30        40        50        60
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TCGGGTATCAACGATGAAATGGACCAGTTGGATCGACTGCCTGCACAACGTTAGGTATGC      10560

CAAAAAAAAGAACACGATCCTTTGCACCCGTTCGATGATTATCAGTATGTTCACAAAAAA      10620

AACTTAAGTTCATCCCAGTGTACAACAGCCCCAACATCTGCCCCAAGTAACAAAAAACAA      10680

CCAATTTATCTTATTCTTATCTGCCACAAAATAATCGGTTTCACACTATTCTCTTGTTAT      10740

ACAAAATTGACAAGTAGGAAGGAGAGGAGTCATCCAAATAAACGGTGCACGTTCTTTGAG      10800

AAAAGTCTTATTTTTCGTAAGATCCAATTTCAACAAACTTTTCTTCAAGTCAAAATTCCT      10860

GATAGTGTATCTCCTCTCGACGACCTCTTGCATTGAACGATCTCCGCTTATCATGAAAAG      10920

TTGCTTGGATAACAAGTATTGCAAGGGGGGGACAGTAGCTATTAAGTTAGTCGGCCCAAG      10980

GAAATGGAGGAGTGATAGTCTCGAATATTATTCACCTCTTTAGCATTACCCGGTCTGGCT      11040

TTAAGGAGTTACGTCTTTTACGCTCGCCAATTTCTTTTTTTAGAATGGTTGGTGTCAAAA      11100

TCGCGAGTTGTGGAAGGTTCAAGTTACTCGATTCGTGATTTTCAAGTATGAGTGGTGAGA      11160

GAGATTCGATATTTTCACGAGGTGTATTCGAGGTCTAGTAGAACGAAGGGTGTCACTAAT      11220

GAAAGTTTCAAGAGTTCATCATCATCTTCTTCTAGTAGATTTTCGCTTTCAAATGAGTAT      11280

GAAAATTCTTCCTCTTTTCTATTGATTTTCTTCATTGTTTTCTTCATTGTTGTGGTTGTT      11340

ATTGAAAAGAAAGAAAATTTATAACAGAAAAAGATGTCAAAAAAAAGGTAAAATGAAAGA      11400

GTATCATATACTTAAAGAGTTGCGTAGAGATAAGTCAAAAGAAACAGAATTATAGTAATT      11460

TCAGCTAAGTTAGAATTC                                                 11478
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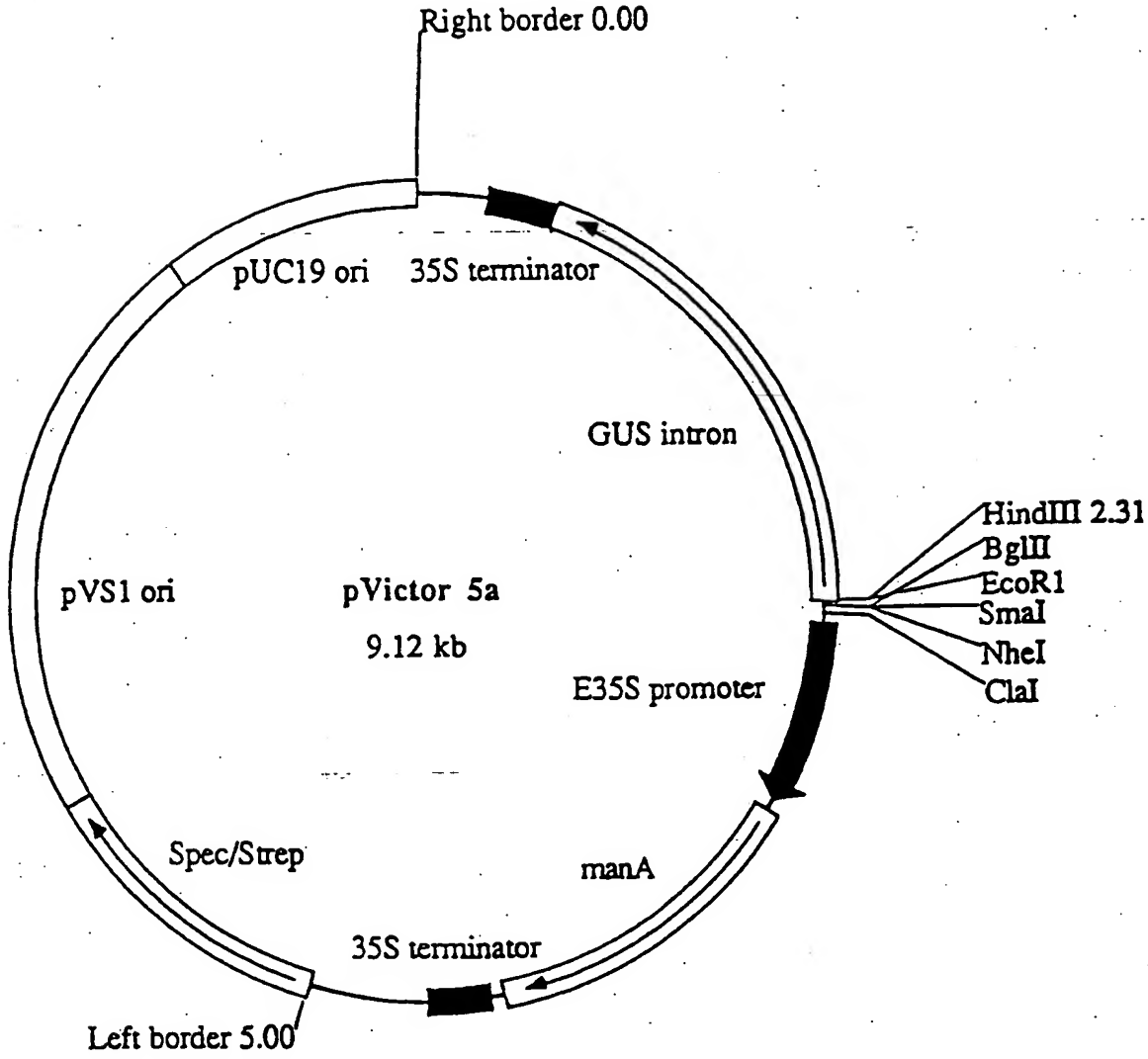
FIG. 8CONTINUED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GUS intron
HindIII 2.31
BglII
EcoR1
SmaI
NheI
ClaI
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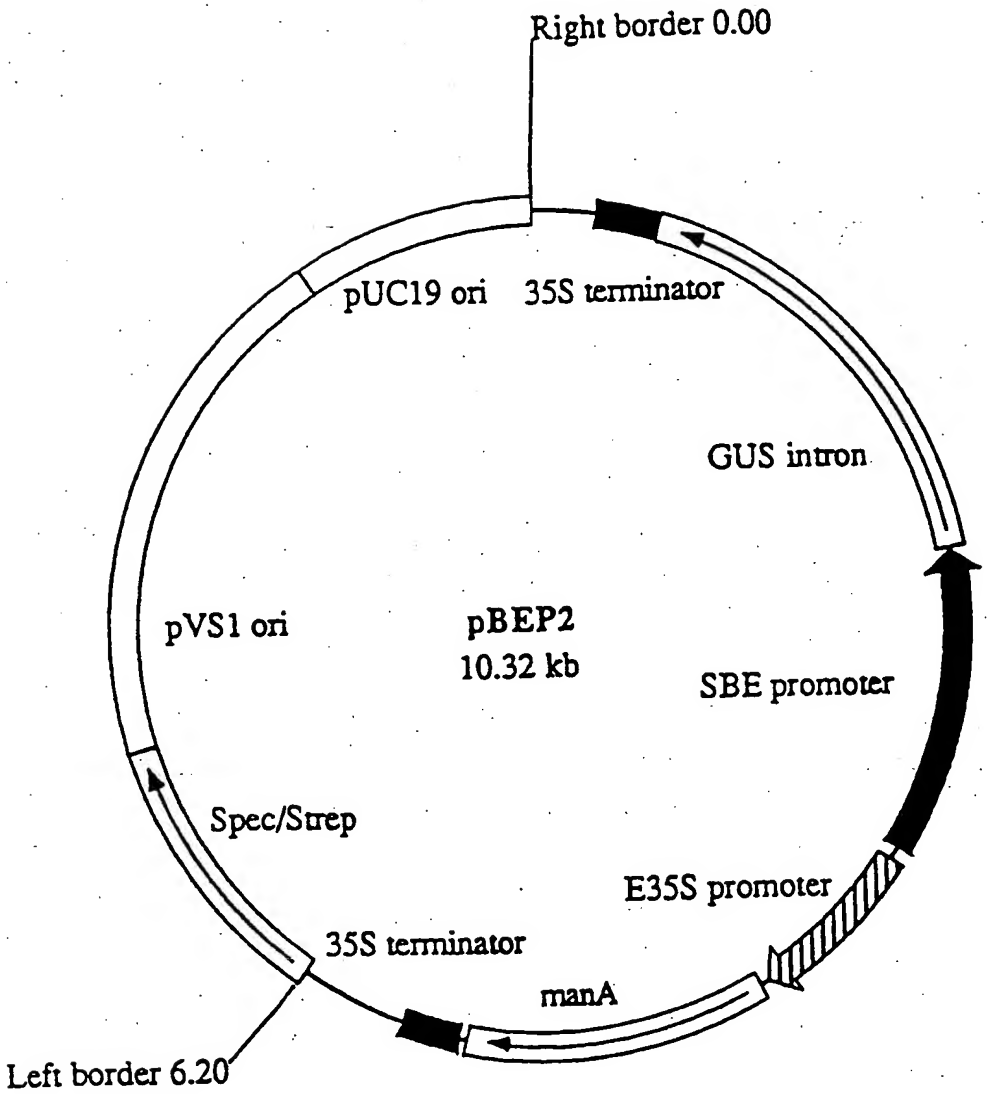
pVictor 5a
9.12 kb
E35S promoter
Spec/Strep
manA
35S terminator
Left border 5.00

FIG. 9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35S terminator
GUS intron
pVS1 ori
pBEP2
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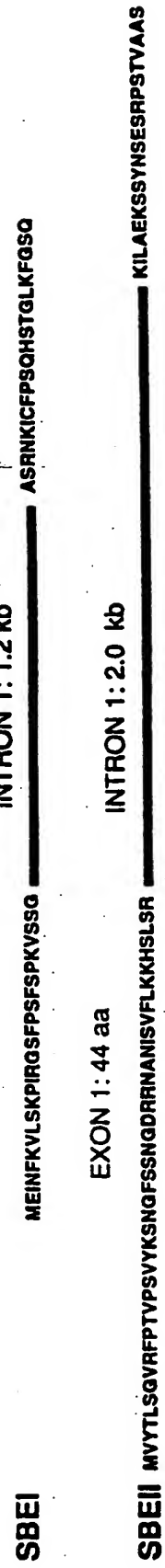
SBE promoter
Spec/Strep
E35S promoter
35S terminator
manA
Left border 6.20

FIG. 10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SBEII
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EXON 1: 44 aa
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INTRON 1: 2.0 kb
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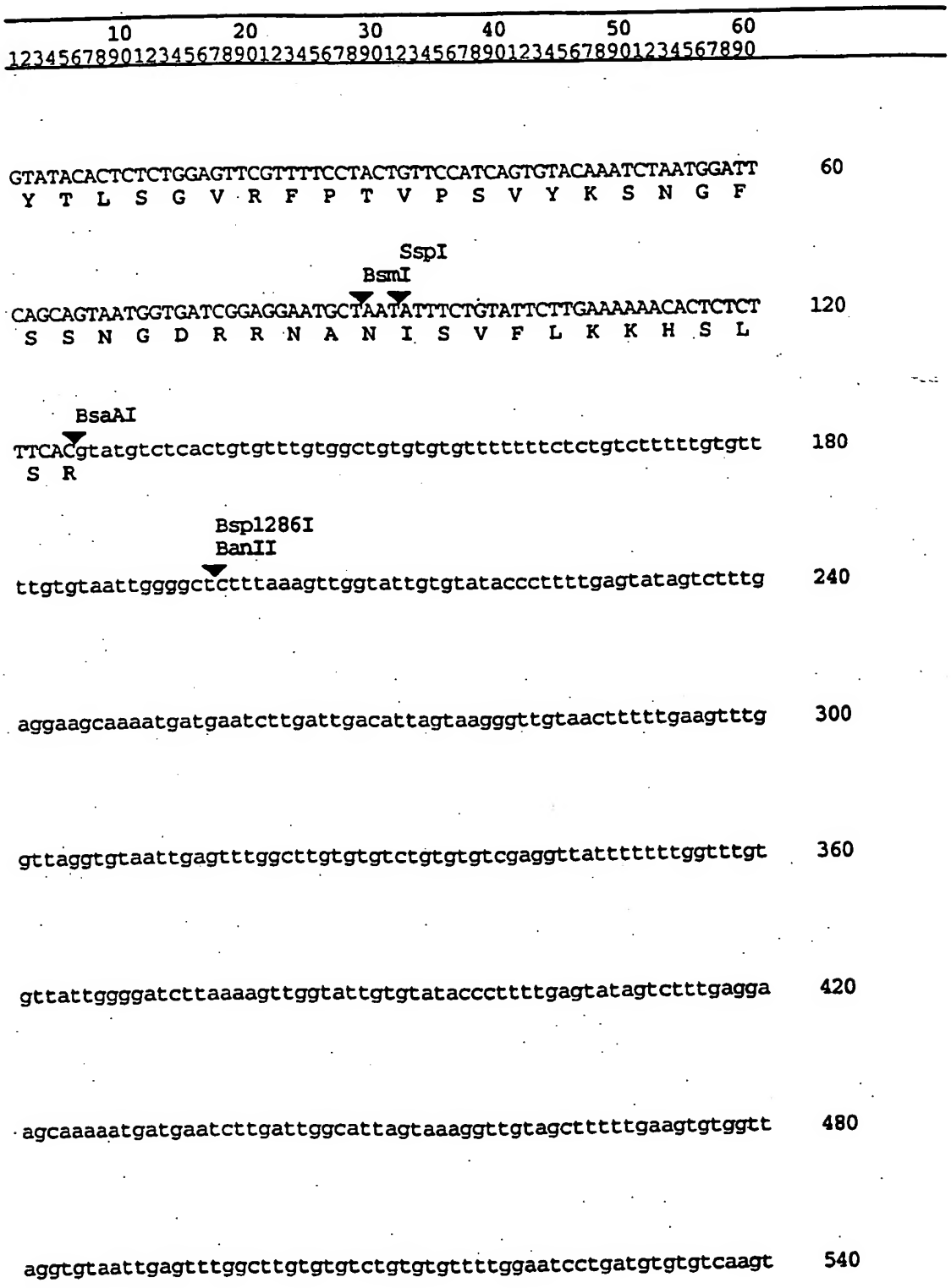
KILAEKSSYNSESRPSTVAAS

FIG. 11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SspI
BsmI
CAGCAGTAATGGTGATCGGAGGAATGCTAATATTTCTGTATTCTTGAAAAAACACTCTCT 120
S S N G D R R N A N I S V F L K K H S L
BsaAI
TTCACgtatgtctcactgtgtttgtggctgtgtgtgttttttttctctgtctttttgtgtt 180
S R
Bspl286I
BanII
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gttaggtgtaattgagtttggcttgtgtgtctgtgtgtcgaggttatttttttggtttgt 360
gttattggggatcttaaaagttggtattgtgtatacccttttgagtatagtctttgagga 420
agcaaaaatgatgaatcttgattggcattagtaaaggttgtagctttttgaagtgtggtt 480
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FIG. 12

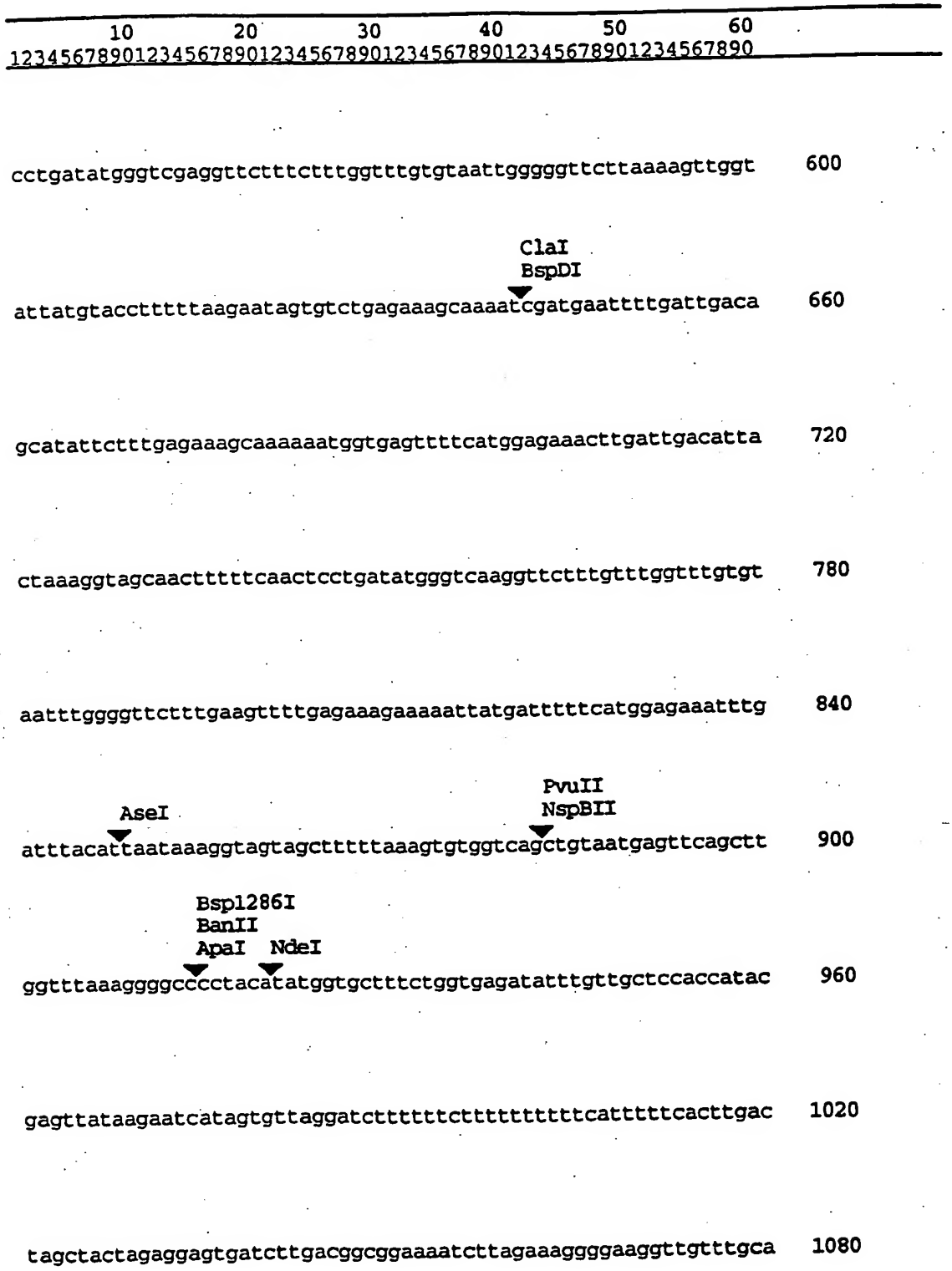

FIG. 12CONTINUED

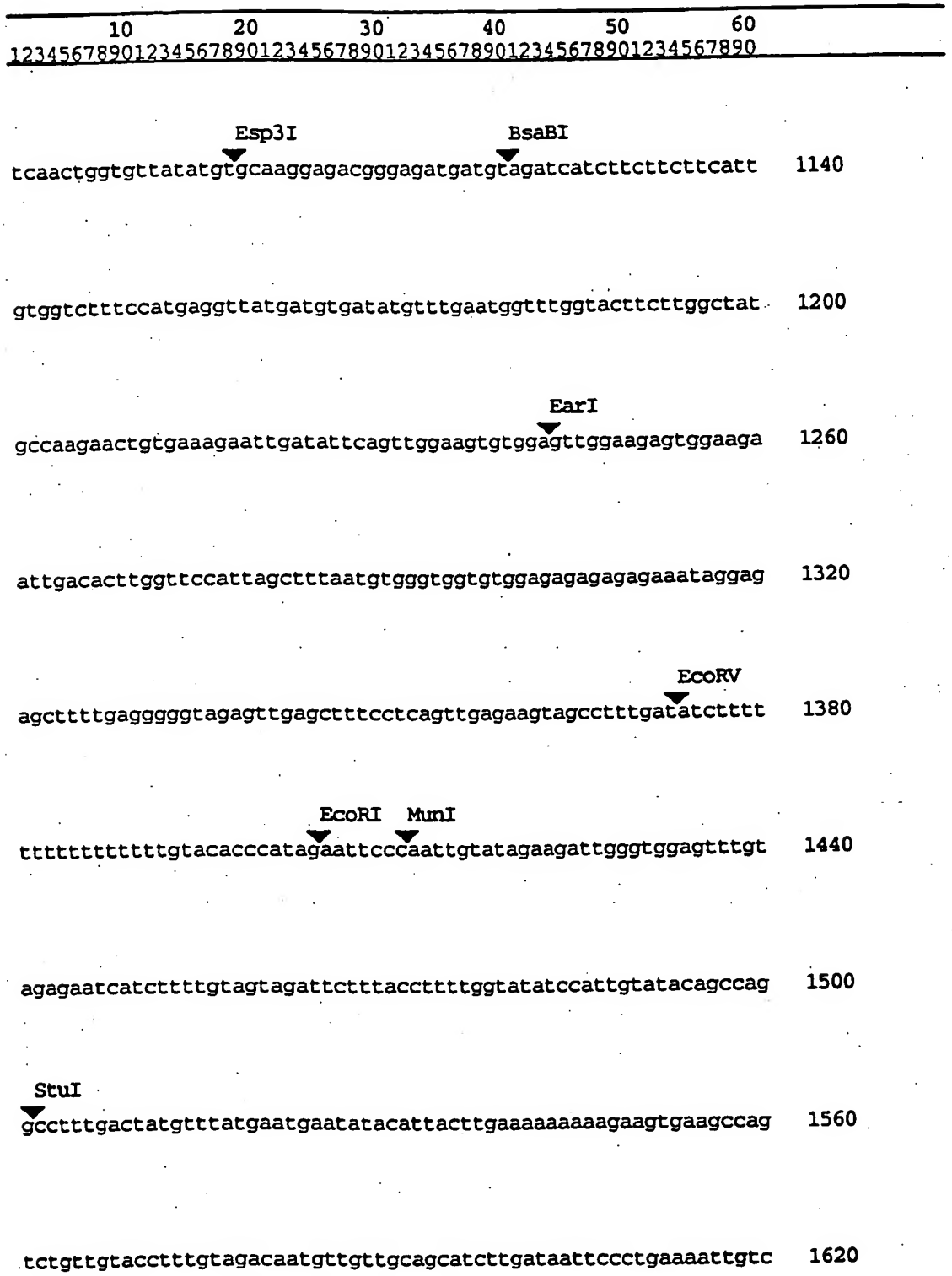

FIG. 12CONTINUED

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ttgaaggccattttaaatcctttgacattgttaaaggtgtttacaagtgttggtctgggt    1740

ttaaaagcacctcttgtatggtgctttctggagtgatctttcttcctccaaaagagaagt    1800

                                        BclI BglII
tgcaagaatcagtgtgtgtacttttttctcttgtatgatcagatctttttttcaatttttc    1860

cgttttagttgatttatccatatagtgaaagttggtgtcatagttgctgtttgtggactt    1920

cctgtaaaagtttttttgatatacttaaaaaattgtcacacagaagaaagagtttttttacc    1980

     AflII
attacttaagctagatgggactgtttgattcttagaccaaataatgaacctttttgttct    2040

     AflIII
cttaacgtgtacttgaaatagtttggtaaaattgtgataggaaaaaagataattcttgat    2100

                                                  EarI
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FIG. 12CONTINUED

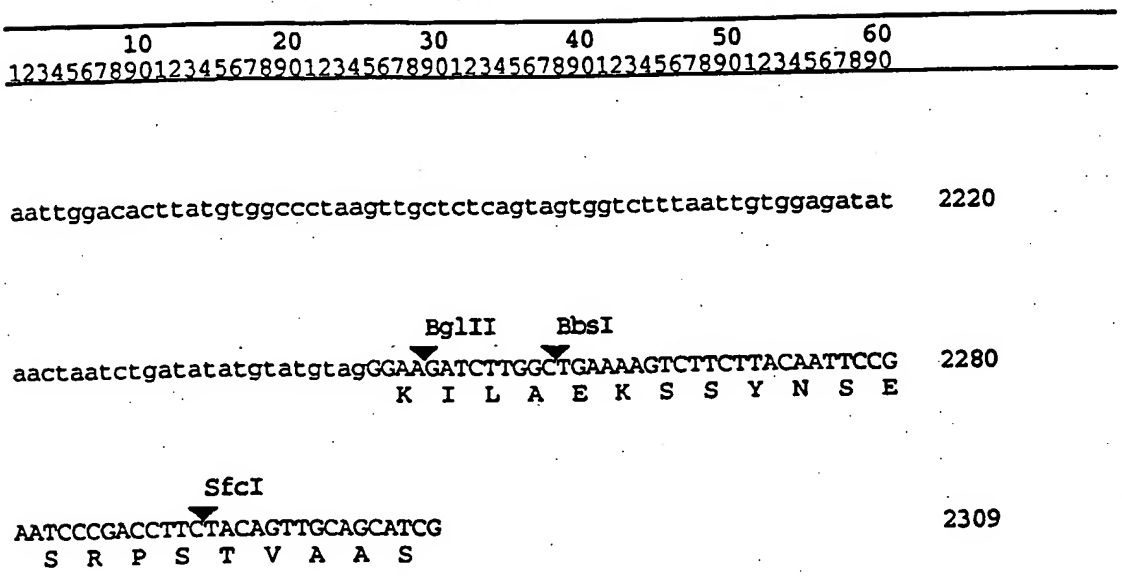
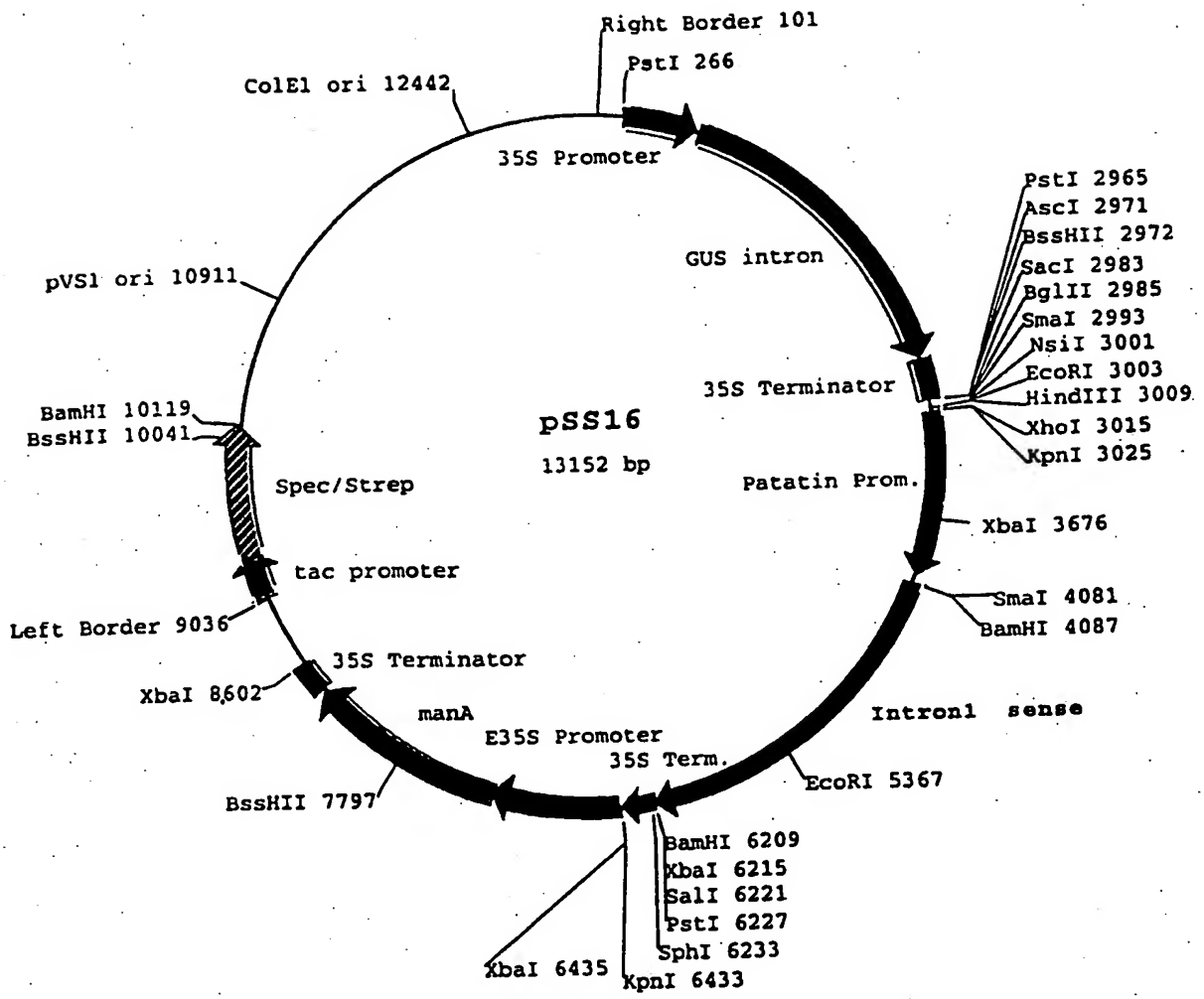


FIG. 12CONTINUED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NsiI 3001
EcoRI 3003
HindIII 3009
XhoI 3015
KpnI 3025
GUS intron
pVS1 ori 10911
35S Terminator
pSS16
13152 bp
BamHI 10119
BssHII 10041
Patatin Prom.
XbaI 3676
Spec/Strep
tac promoter
SmaI 4081
BamHI 4087
Left Border 9036
35S Terminator
XbaI 8602
manA
Intron1 sense
E35S Promoter
35S Term.
EcoRI 5367
BssHII 7797
BamHI 6209
XbaI 6215
SalI 6221
PstI 6227
SphI 6233
XbaI 6435
KpnI 6433

FIG. 13

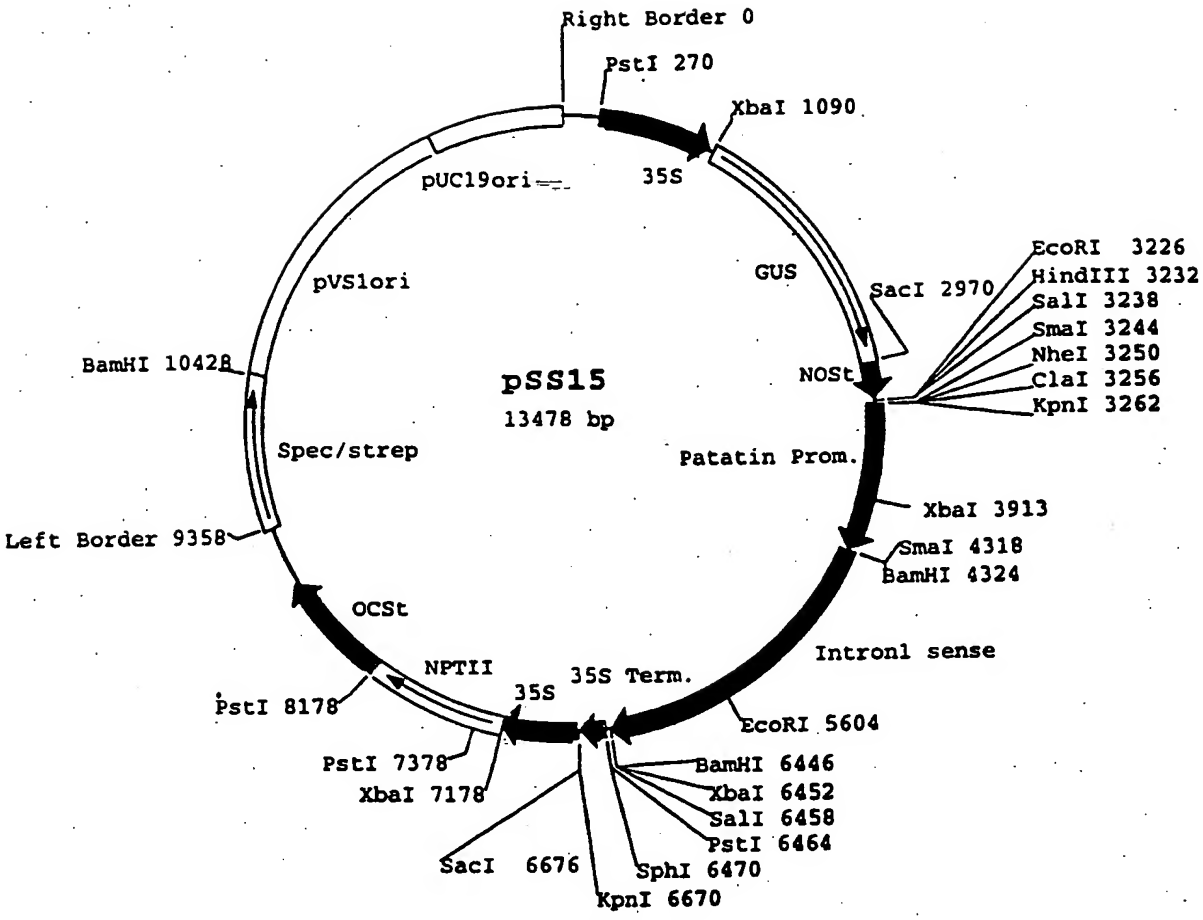
Right Border 0
PstI 270
XbaI 1090
35S
pUC19ori
GUS
EcoRI 3226
HindIII 3232
SalI 3238
SmaI 3244
NheI 3250
ClaI 3256
KpnI 3262
SacI 2970
NOSt
pVS1ori
BamHI 10428
pSS15
13478 bp
Patatin Prom.
Spec/strep
XbaI 3913
SmaI 4318
BamHI 4324
Left Border 9358
Intron1 sense
OCSt
NPTII
35S Term.
35S
PstI 8178
EcoRI 5604
PstI 7378
XbaI 7178
BamHI 6446
XbaI 6452
SalI 6458
PstI 6464
SacI 6676
SphI 6470
KpnI 6670

FIG. 14

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/IB 98/00295

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/82 C12N9/10 C12N15/11 C08B30/04

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C08B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 04112 A (DANISCO ;POULSEN PETER (DK)) 6 February 1997 cited in the application see the whole document ---	1-19
X	WO 97 04113 A (DANISCO ;POULSEN PETER (DK)) 6 February 1997 cited in the application see the whole document ---	1-19
Y	WO 96 34968 A (NAT STARCH CHEM INVEST ;COOKE DAVID (GB); DEBET MARTINE (GB); GIDL) 7 November 1996 cited in the application see page 5, paragraph 3 - paragraph 4 see page 9, paragraph 2 - page 10, paragraph 1	1-19
X	see page 11, paragraph 3 ---	16-18
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 June 1998	09/06/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Chakravarty, A

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Inte onal Application No
PCT/IB 98/00295

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category '	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 90 12084 A (DNA PLANT TECHN CORP) 18 October 1990 see page 9, line 14 - line 19 see page 11, line 25 - page 12, line 11	1-19
Y	WO 92 15680 A (UNIV TEXAS) 17 September 1992 see page 6, line 17 - line 28	1-19

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[illegible]nal Application No
PCT/IB 98/00295

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